Micropropagation and Horticultural Potential of Native Tasmanian Liliaceae and Iridaceae

Volume 1

by

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"Sleigh bells in northern lands,

Where Christmas comes with snow,

But flower bells in Australia,

Where Christmas wildflowers grow."

(Anon.)

Declaration

This thesis contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief, no material previously published or written by another person, except where due acknowledgement is made in the text of the thesis.

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Abstract

A study encompassing a range of botanical research areas was undertaken to determine the horticultural potential of some members of the native Tasmanian Liliaceae and Iridaceae. Six main species were studied: *Blandfordia punicea*, *Dianella tasmanica* and *Milligania densiflora* from the Liliaceae as well as *Diplarrena moraea*, *Diplarrena latifolia* and *Isophysis tasmanica* from the Iridaceae. With the exception of *D. tasmanica* and *D. moraea*, all species are endemic to Tasmania.

Seed germination trials were carried out to assess the viability of this method of propagation. The natural germination percentages were extremely high for *B. punicea* (93-100%), *D. moraea* (97%) and *I. tasmanica* (90-100%) and reasonably high for *D. latifolia* (76%). For *D. tasmanica* and *M. densiflora* they were much lower (38% and 48%, respectively). The germination percentage of *M. densiflora* could be increased to 88% simply by germinating the seed *in vitro*; it could be increased further to 90% by disinfesting seeds for 35 min and growing them on a 1/2 MS medium. However, for *D. tasmanica* there are dormancy mechanisms present which proved more difficult to overcome. The most promising methods for this species were smoke treatments and partial removal of the testa. The percentage of seeds germinating increased with increasing smoke extract concentration; however, 100% smoke inhibited germination. Removal of part of the testa and growth *in vitro* was the best treatment, with 90% germination achieved.

In vitro propagation was achieved for all species studied. Successful protocols were devised for disinfestation, shoot initiation and multiplication, rooting and transplanting to *ex vitro* conditions. Not all explant types investigated were suitable for all species, with the major problem being contamination. Meristematic regions originating underground were more difficult to disinfest than the immature floral explant types that originated above-ground. In general, a MS or 1/2 MS medium was suitable for growth of all species studied and BAP (at 8-32μM) was generally successful for shoot multiplication. Roots often grew on shoots on multiplication

media, or could be induced to form on a medium containing NAA (or less successfully, IAA or IBA) or by placing them on a medium free of plant growth regulators. Transplanting was successful for all species, with a high percentage of plantlets surviving. However, they were susceptible to damping off if too much water was applied.

Experiments were also performed to examine the possibility of forcing *D. tasmanica* plants to flower out-of-season. By manipulation of temperature and photoperiod plants could be forced to flower earlier. The fire-related treatments of smoke and foliage removal also increased the percentage of flowering plants compared to the control.

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Table of Contents

Volume 1

Volume 2

Volume 3

List of Tables

Volume 1

Volume 2

Volume 3

List of Figures

Volume 1

Volume 2

Volume 3

List of Plates

Volume 1

Volume 2

Volume 3

List of Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic Acid
2iP	6-(γ,γ-dimethylallylamino) Purine
AC	Activated Charcoal
ACT	Australian Capital Territory
ANBG	Australian National Botanic Gardens
ANOVA	Analysis of Variance (statistical technique)
AR1	*D. tasmanica* Arve Road plant 1
AR2	*D. tasmanica* Arve Road plant 2
AR3	*D. tasmanica* Arve Road plant 3
B5 medium	Gamborg *et al.* (1968)
BAP	6-Benzylaminopurine
$CaCl_2.2H_2O$	Calcium chloride dihydrate
$(CS(NH_2))_2$	Thiourea
$CuSO_4.5H_2O$	Cupric sulphate pentahydrate
DAA	Days After Application
DAS	Days After Sowing
DN	Day-Neutral plant
EC medium	Embryo Culture medium
EDTA	Ethylenediaminetetraacetic Acid
EtOH	Ethanol
FB	Flower Bud
FBM	Flower Bud (initiation) Medium
FBMM	Flower Bud Multiplication Medium

$FeCl_3$	Ferric chloride
$FeSO_4.7H_20$	Ferrous sulphate heptahydrate
FSS	Floral Scape Section
GA/GA_3	Gibberellic Acid
H_3BO_3	Boric Acid
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
HSD	Honestly Significant Difference (statistical term)
IAA	Indole-3-Acetic Acid
IBA	Indole-3-Butyric Acid
ID	Intermediate-Day plant
KH_2PO_4	Monopotassium phosphate
KI	Potasssium iodide
KIN/Kinetin	6-furfurylaminpurine
KNO_3	Potassium nitrate
LB1	*D. tasmanica* Lime Bay plant 1
LD	Long-Day plant
LR1	*D. tasmanica* Lidgerwood Rd plant 1
LR2	*D. tasmanica* Lidgerwood Rd plant 2
LS medium	Linsmaier and Skoog medium (1965)
LSD	Long-Short-Day plant
LSV	Lily Symptomless Virus
M	Molar (concentration)
MBG	Melbourne Botanic Gardens
$MgSO_4.7H_2O$	Magnesium sulphate heptahydrate

Min/s	Minute/s
mM	millimolar (concentration)
$MnSO_4.4H_2O$	Manganese sulphate tetrahydrate
MS medium	Murashige and Skoog medium (1962)
NA_2EDTA	Ethylenediamine Tetraacetic Acid Disodium salt
$NA_2MoO_4.2H_2O$	Sodium molybdate dihydrate
NAA	α-Naphthaleneacetic Acid
NaFeEDTA	Ferric sodium salt
NH_4NO_3	Ammonium nitrate
NM medium	*Narcissus* Multiplication medium
NN medium	*Narcissus* initiation medium
NaOCl	Sodium hypochlorite
no.	number
n.s	not significant (statistically)
NSW	New South Wales
PBR	Plant Breeders' Rights
PGR	Plant Growth Regulator
p.p	photoperiod
ppm	parts per million
Qld	Queensland
SC1	Mature *B. punicea* seed lot
SC2	Intermediate (& immature) *B. punicea* seed lot
SC3	Immature *B. punicea* seed lot
SD	Short-Day plant
SDW	Sterile Distilled Water

SLD	Short-Long-Day plant
sp.	Species
SW	South West
TCL	Thin Cell Layer
μM	micromolar
VFT	Venus fly trap medium
v/v	volume/volume
WA	Western Australia
w/v	weight/volume
$ZnSO_4.7H_2O$	Zinc sulphate heptahydrate

Chapter 1
General Introduction

1.1 Introduction

Overseas interest in Australia's native flora began soon after Europeans arrived on the continent (Stewart, 1999a). Botanists such as Sir Joseph Banks made extensive collections of the "New Holland" flora, which stimulated great interest and amazement due to its uniqueness (Cameron, 1992; Johnson and Burchett, 1996; Wrigley and Fagg, 1996; Stewart, 1999a; Moody, 2002). Horticulture was a very popular pastime for the landed gentry of Europe in the late 18th century and it actually became a status symbol to grow and flower these plants from the newly discovered lands of the southern hemisphere (Stewart, 1999a). By 1800 there were 170 cultivated Australian species in Britain (Johnson and Burchett, 1996) and Banksias, Acacias and Eucalypts were offered for sale in exclusive nursery catalogues, fetching a small fortune (Stewart, 1999a).

In contrast, the early Australian settlers saw the native flora as strange and unfamiliar (Johnson and Burchett, 1996; Wrigley and Fagg, 1996; Barnard *et al.*, 2004). Native trees were heavily exploited for timber and bushland was extensively cleared (Moody, 2002) so that gardens containing roses, carnations, oaks, elms and other European species could be planted (Johnson and Burchett, 1996; Wrigley and Fagg, 1996).

In the early 1900s some gardeners began to look more closely at the local flora and appreciate its unique beauty (Moody, 2002) but cultivation was not generally popular (Wrigley and Fagg, 1979; Wrigley, 1988) as the plants were regarded as being difficult to propagate and cultivate (Johnson and Burchett, 1996).

After the Second World War there was a resurgence in interest in the native flora (Johnson and Burchett, 1996) and in the mid 1950s the Society for Growing Australian Plants was formed by native plant enthusiasts (Johnson and Burchett, 1996; Wrigley and Fagg, 1996; Moody, 2002; Barnard *et al.*, 2004). This organisation has played a major role in encouraging the use of native plants as garden subjects (Johnson and Burchett, 1996; Wrigley and Fagg, 1996; Barnard *et al.*, 2004).

Since the end of the 1950s the popularity of Australian native plants has steadily increased, and, especially in the last 30 years, this interest has accelerated dramatically to the boom that is being experienced today (Wrigley and Fagg, 1996). As more and more Australian gardeners choose native plants instead of exotic species (Blombery, 1977; Lamont, 1988; Blombery and Maloney, 1994), the horticulture industry has also increased its interest in native plants to meet this demand (Wrigley and Fagg, 1996; Moody, 2002; Stewart, 2002). Although the horticulture of Australian plants is still in its infancy (Johnson and Burchett, 1996), within the last 20 years a huge amount of work has seen the improvement of many Australian plants through selection of superior strains and hybridisation to create new cultivars (Stewart, 1999a). The advent of Plant Breeders Rights (PBR) has also probably helped in increasing the number of new cultivars available as there is a monetary incentive for undertaking research to develop them (Wrigley and Fagg, 1996).

The fact that the industry is still in its infancy means that there are endless opportunities for commercial producers of cut flowers and ornamental flowering pot and garden plants to develop new crops (Stewart, 1999a). Within the last 20 years the potential of many native species, including several monocotyledons, has been realised and these are now produced in commercial quantities for both the domestic and overseas markets. For example, the "Kangaroo Paws" (*Anigozanthos* and *Macropidia*) are one of the top three native flowers to be exported from Australia (Considine, 1993; Parliament of Victoria, 2000). Approximately 7 million flower stems from these species were produced in Australia in 1993, 96% of which were from commercial plantations, the remainder being "bush-picked" (i.e. harvested from native populations) (Worrall, 1996a). Kangaroo Paws are generally exported as cut flowers to Japan, the USA, Europe and, increasingly, other South-East Asian

countries, but there is also a major market for flowering pot plants, especially in Europe, Japan and the USA (Worrall, 1996a). *Anigozanthos* is also a major export crop for Israel (Johnson and Burchett, 1996), where research is being undertaken into the breeding and development of new varieties (Worrall, 1996a; Parliament of Victoria, 2000). Hybrids of *Anigozanthos flavidis* are most commonly grown (Worrall, 1996a; Parliament of Victoria, 2000), but the black kangaroo paw, *Macropidia fuliginosa*, is the most highly sought-after (Parliament of Victoria, 2000). There has been a lucrative market for this species as a fresh cut flower in Europe and North America for many years (Tan and Vlok, 1989). Another example is the "Christmas Bell" genus, *Blandfordia*. Within Australia, *Blandfordia* has been a popular cut flower for many years, but has only been in cultivation since the early 1990s (Johnson, 1996a). Prior to this the supply of blooms came from licensed harvesting from bushland areas (Johnson, 1987, 1990, 1993, 1996; Johnson and Burchett, 1991; Gorst, 1996; Wrigley and Fagg, 1996). *B. grandiflora*, the most outstanding species in terms of floral attributes (Johnson, 1996a; Stewart, 2002; Page and Olds, 2004) is now being grown commercially for the domestic and overseas market as a cut flower (Gorst, 1996; Johnson, 1996a). Blooms have been exported to Japan every year since 1991, and more recently to Holland in 1993 (Johnson, 1996a). There has also been interest from growers in New Zealand, Israel and Egypt (Johnson, 1987).

One of the newest additions to the export trade, the "Gymea Lily" or "Giant Lily", *Doryanthes excelsa*, is currently experiencing an increase in demand both on local and overseas markets (Stewart, 1999a; Smith, 2000). This monocot is a spectacular feature plant (Greig, 1993; Wrigley and Fagg, 1996) with its massive flower spike reaching 8 (Smith, 2000) or even 10 metres in height (Stewart, 2002). The foliage is well suited to modern buildings and landscape design and the extremely long-lasting cut flowers (Greig, 1993) are highly sought-after by floral designers who use them as feature flowers for large, imposing arrangements in hotel foyers (Smith, 2000). Unfortunately, the majority of flower stems are currently being supplied from bush-picking, with only a small number of stems coming from commercial row production (Smith, 2000). As the cut flowers afford very high returns, fetching up to $300 in

Japan (Stewart, 1999a), stems are even being illegally removed from roadsides, private properties and national parks (Smith, 2000).

Associated with this increase in popularity of native plants is a need for the expansion of the botanical knowledge of the species involved, to ensure that they are preserved in their natural habitats and that the end products are of the highest quality. The supply of flowers from bush-picking (as mentioned above) can have disastrous effects on native plant species and their habitats. When harvesting from the bush the largest and most outstanding blooms would naturally be selected (Stewart, 1999a). The continual removal of the best blooms would deplete the gene pool (Stewart, 1999a) as well as the seed bank, and thus has implications for the future regeneration of the species involved (Plummer, 1996; Sedgley, 1996). As well as the problems associated with the actual picking of the blooms from the wild, there are also consequences for the surrounding area. Access vehicles can cause disturbance via soil and plant destruction, and soil-borne diseases can be spread on tyres and footwear. Diseases can also be spread when cutting the blooms from the plant, on infected secateurs (Sedgley, 1996). A number of native species have already been adversely affected by the associated effects of bush-picking. *Pimelea physodes* has been bush-picked to the brink of extinction in natural populations and is still not under cultivation (Slater, 1996); *Banksia*, which is still the second largest bush-picked genus, has been threatened by the spread of the root rot fungus *Phytopthora cinnamomi* (Sedgley, 1996). A number of native monocot species have also been under threat from bush-picking, namely *Anigozanthos manglesii* and *Macropidia fuliginosa* (Stewart, 1999a). *Blandfordia* has also become locally extinct in some mainland areas due to this practice (Johnson and Burchett, 1996).

Bush-picked blooms are also usually of inferior quality to those produced under cultivation (Sedgley, 1996; Worrall, 1996a; Stewart, 1999a) and can often have a negative impact on the market (Worrall, 1996a), especially the export market, which has particularly high standards (Sedgley, 1996). For at least one genus that is exported to Japan (*Blandfordia)*, only flowers which have been cultivated are of export quality and can meet the phytosanitary requirements of the country (Johnson, 1996a). Although there are so many negative aspects associated with bush-picking, it

is recognised that it will still be a source of commercial quantities of many plant species until their propagation and cultivation requirements are fully understood (Johnson and Burchett, 1996). It is encouraging to see that for at least some native species, especially those that have been cultivated for some time, the percentage of flowers that is being supplied from bush-picking is decreasing. For example, the cultivation of new, improved hybrid kangaroo paws which have a higher quality of blooms and yield a uniform, long-lasting crop over a longer time period, has caused a substantial decline in bush-picking (Stewart, 1999a). Only 4 % of kangaroo paws were being harvested from native populations in 1996 and this percentage was continuing to decline (Worrall, 1996a). The wildflower industry in Western Australia (WA), which was historically based almost entirely on bush-picking, has also been pushed towards cultivation due to conservation concerns, market demand for high quality products and consistency of supply (Parliament of Victoria, 2000). In 1995 - 1996 about 15% of WA production came from bush-picking, a significant decrease from the figure of 50% ten years previously in 1985 (Parliament of Victoria, 2000).

A similar problem to bush-picking, and one to which the native monocots are particularly susceptible, is the removal of entire plants from their natural habitats. The native monocots are perhaps one of the least studied and potentially most endangered groups of native plants (Dr. Ron Crowden, pers. comm., 1995). The reason for this is that they possess underground storage organs such as bulbs, corms, tubers and rhizomes and therefore, as the meristematic areas are subterranean, in order to propagate them they must be excavated. They cannot be grown from cuttings (Wrigley and Fagg, 1996) and if clones are required seed is not an option. The practice of removing geophytic plants from their natural habitats has become a problem in many other parts of the world including Japan, Portugal and Turkey (Read, 1989; Thompson Campbell, 1989; Read and Thomas, 1992). In many cases the storage organs are collected to be directly exported to other countries, rather than for propagation purposes, and a number of geophyte species are now extinct as a result of such over-exploitation. For example, *Tecophilea cyanocrocus* is now extinct from its natural habitat in Chile due to the collection of its bulbs from the wild (Read, 1989) and thousands of bulbs of *Sternbergia candida* were "on the market"

within two years of its discovery as a new species. It is unknown whether any plants remain at its only confirmed locality (Read, 1989). Fortunately, problems have not occurred to this extent in Australia. However, as mentioned previously, many Australian native plants which are difficult to propagate using conventional methods have been, or still are, being collected from their natural habitats, and it is necessary to ensure that this practice does not continue, or is at least sustainable. At present the collection of native plants and plant parts can only be done legally via permits or licences issued by the various state national park services, or other authorities (Johnson and Burchett, 1996) and in some cases legislation has been introduced to prevent the collection of rare and/or threatened species. For example, *Banksia coccinea* and *B. baxteri* can no longer be legally bush-picked from Crown land, due to legislation that was introduced (Sedgley, 1996). However, before legislation like this can be enforced it is necessary for alternative methods of propagation to be available (Sward, 1995). Relatively new technologies such as plant tissue culture and embryo rescue are now being routinely used to propagate recalcitrant species (Stewart, 1999a) and the use of smoke treatments for promoting the germination of difficult seed has allowed some new species to be brought into cultivation (Wrigley and Fagg, 1996). Once the knowledge of how to propagate and cultivate these plants is available they can then be produced in commercial quantities and wild populations can be conserved.

There are a number of native Tasmanian genera within the Liliaceae and Iridaceae families that have been suggested as having potential for horticultural uses. Some of these are already used within the horticulture industry, but generally the species in use are mainland ones, rather than those from Tasmania. The genera of note include *Blandfordia*, *Dianella* and *Milligania* (Liliaceae) as well as *Diplarrena* and *Isophysis* (Iridaceae).

1.1.1 *Blandfordia*

The genus *Blandfordia* is endemic to subtropical and temperate regions of eastern Australia (Henderson, 1987a; Collier, 1991; Stanley and Ross, 1989; Curtis and Morris, 1994; Greig, 1999). It was named in honour of the Marquis of Blandford, George Spencer Churchill (1766-1840), for his enthusiastic practice of horticulture in Reading, England (Henderson, 1987a; Greig, 1990). It contains 4 species: *B. grandiflora*, "Northern Christmas Bell", which occurs in north eastern NSW and south eastern Qld (Conabere and Garnet, 1987; Henderson, 1987a; Wrigley and Fagg, 1996; Greig, 1999), *B. nobilis*, "Christmas Bell", endemic to damp sandstone areas in NSW (Conabere and Garnet, 1987; Henderson, 1987a; Wrigley and Fagg, 1996; Greig, 1990, 1999), *B. cunninghamii*, "Mountain Christmas Bell", also endemic to NSW, occurring in the Blue Mountains (Conabere and Garnet, 1987; Henderson, 1987a; Johnson, 1996a; Wrigley and Fagg, 1996; Greig, 1990, 1999; Barnard *et al.*, 2004) and *B. punicea*, "Tasmanian Christmas Bell", which is endemic to Tasmania (Conabere and Garnet, 1987; Henderson, 1987a; Collier, 1991; Cameron, 1992; Curtis and Morris, 1994; Gorst, 1996; Johnson, 1996a; Wrigley and Fagg, 1996; Kirkpatrick, 1997; Greig, 1999; Barnard *et al.*, 2004) (Plate 1.1).

Plate 1.1. *Blandfordia punicea* flowering plant. Scale bar = 2.5 cm.

Blandfordia is undoubtedly the most well known Australian Liliaceae genus overseas. Indeed the horticultural potential of the genus was recognised in England as early as 1803, when *B. nobilis* entered cultivation as a glasshouse plant (Greig, 1990; Johnson, 1996a). They are also cultivated in other areas of Europe and North America (Stanley and Ross, 1989; Henderson, 1987a). As mentioned previously, *Blandfordia* species are highly successful cut flowers both within Australia and as an export crop (Gorst, 1996; Johnson, 1996a). They are also suitable as pot plant specimens (Blombery, 1972; Moore *et al.*, 1993; Johnson, 1996a; Stewart, 2002), as a rockery plant (Moore *et al.*, 1993; Wrigley and Fagg, 1996), grown in drifts (Greig, 1990, 1993) or mass plantings (Greig, 1993).

Blandfordia can be propagated relatively easily by seed (Blombery, 1972; Johnson, 1987; Collier, 1991; Greig, 1993; Moore *et al.*, 1993; Wrigley and Fagg, 1996; Stewart, 1999b; 2002; Page and Olds, 2004) or by division of the rhizomatous corm (Johnson, 1996a; Stewart, 1999b; Page and Olds, 2004). The plants require a well drained soil (Greig, 1993; Wrigley and Fagg, 1996; Barnard *et al.*, 2004), ample moisture (Greig, 1993; Johnson, 1996a; Wrigley and Fagg, 1996; Barnard *et al.*, 2004; Page and Olds, 2004) especially at budding and flowering (Johnson, 1996a) and full sun to partial shade (Greig, 1993; Wrigley and Fagg, 1996; Page and Olds, 2004). In warm areas *B. punicea* requires some shade (Wrigley and Fagg, 1996). Although *Blandfordia* naturally grows in low nutrient soils, it actually grows much better when fertilised with a suitable regime, such as a slow release fertiliser together with regular applications of liquid fertiliser at low concentrations (Johnson, 1996a). The pH of the soil must be in the acidic range, 5.0-5.5, and lime and/or dolomite can be used to achieve this (Johnson, 1996a). *Blandfordia* can be difficult to maintain in cultivation as it is prone to root-rot and sensitive to nutrient imbalances (Page and Olds, 2004).

1.1.2 *Dianella*

The *Dianella* genus consists of 25-30 species which occur in south eastern Africa, south east Asia, the Pacific islands including Hawaii, New Zealand and Australia (Henderson, 1987b; Stanley and Ross, 1989; Greig, 1990; Wilson, 1993; Conran,

1994; Curtis and Morris, 1994; Wrigley and Fagg, 1996). It is named after "Diana", Roman goddess of the hunt and queen of the woods, and the suffix "ella" meaning small (Elliot and Jones, 1984; Henderson, 1987b; Greig, 1990; Page and Olds, 2004). There are fifteen species in Australia, eleven of which are endemic (Henderson, 1987b; Stanley and Ross, 1989; Wilson, 1993; Wrigley and Fagg, 1996). According to Curtis and Morris (1994) four species occur in Tasmania, *D. tasmanica*, *D. revoluta*, *D. longifolia* var. *longifolia* and *D. brevicaulis*. *D. brevicaulis* was previously named *D. revoluta* var. *brevicaulis*, but has been raised to specific ranking following a study by Carr and Horsfall (1995). *D. amoena*, a relatively new species described by Carr and Horsfall (1995), previously classified as either *D. tasmanica* or *D. caerulea* var. *caerulea*, has also been noted as occurring in the Midlands area of Tasmania.

Dianellas occur across all states of Australia in a wide range of habitat types including rainforest, semi-desert areas, coastal sand-dunes, inland ranges and tablelands (Elliot and Jones, 1984). They are hardy plants (Elliot and Jones, 1984; Wrigley and Fagg, 1983; 1996), especially *D. revoluta* (Stewart, 2002), which are able to survive extremes of temperature, dryness and bushfires due to their extensive subterranean rhizomes and root system (Elliot and Jones, 1984). Dianellas are extremely well suited to cultivation (Elliot and Jones, 1984; Wrigley and Fagg, 1996). Their growth habit of tussocks or spreading colonies means that they are particularly useful for rockeries (Elliot and Jones, 1984; Wrigley and Fagg, 1983, 1996), especially those associated with water features due to their large flax-like leaves (Wrigley and Fagg, 1983, 1996). They are also useful for breaking up lines of shrubbery and as a container plant (Elliot and Jones, 1984). *D. caerulea* has been suggested as a long-lasting cut flower (Greig, 1993) and the long flax-like leaves have strong fibres which make them suitable for basket weaving (Greig, 1990). The fibrous leaves were also used by the Aborigines (Fairley and Moore, 1989). Another possible use of this genus is as a bush food. Although there have been reports that the berries are poisonous (Hurst, 1942; Everist, 1974) most are circumstantial and inconclusive (Everist, 1974). Recipes are available for *Dianella* berry jam, and the fruits may have other culinary uses.

For cultivation, most conditions are satisfactory (Wrigley and Fagg, 1983, 1996). They are fairly adaptable to a range of drainage conditions and soil types (Stewart, 2002) but will do best in a moderately fertile, humus-rich, well-drained, neutral to acidic soil (Page and Olds, 2004). Some species (for example *D. tasmanica)* grow well in shady situations (Plate 1.2), while most flower best in an open sunny area (Elliot and Jones, 1984). Once established the plants are long-lived and require very little attention. *Dianella* is one of the few genera with blue flowers and although individual flowers are reasonably short-lived they open in succession; thus flowers are present over a long time period (Elliot and Jones, 1984). According to Elliot and Jones (1984) a tremendous variation occurs within most species which means that a large range of forms are available for garden culture. These can be maintained by vegetative propagation in the form of division (Barnard *et al*., 2004; Page and Olds, 2004). Despite the wide range of species available, until recently only three, *D. caerulea, D. revoluta* and *D. tasmanica*, appeared to be sold by a small number of native plant nurseries throughout Australia in Tasmania, Victoria, New South Wales and Queensland (Hutchison, 1993).

Plate 1.2. *D. tasmanica* plants thriving in a shady garden area. Scale bar = 20 cm.

Dianella has become very popular in the last few years, with the release of the "Border" series™ of *D. ensifolia* by Anthony Tesselaar International on the 1st of March 2002 (Plate 1.3). There are 3 different varieties: "Border Gold", "Border Silver" and "Border Emerald" all with variegated foliage. As the name suggests, these varieties make excellent border plants and they are also suitable as container plants, both indoors and out (Gardening Australia, 2002). The plants reach about 60 cm in height, are drought-tolerant and low maintenance (Better Homes and Gardens, 2002; Gardening Australia, 2002). It is hoped that the popularity of these new varieties will also lead to interest in the other *Dianella* species.

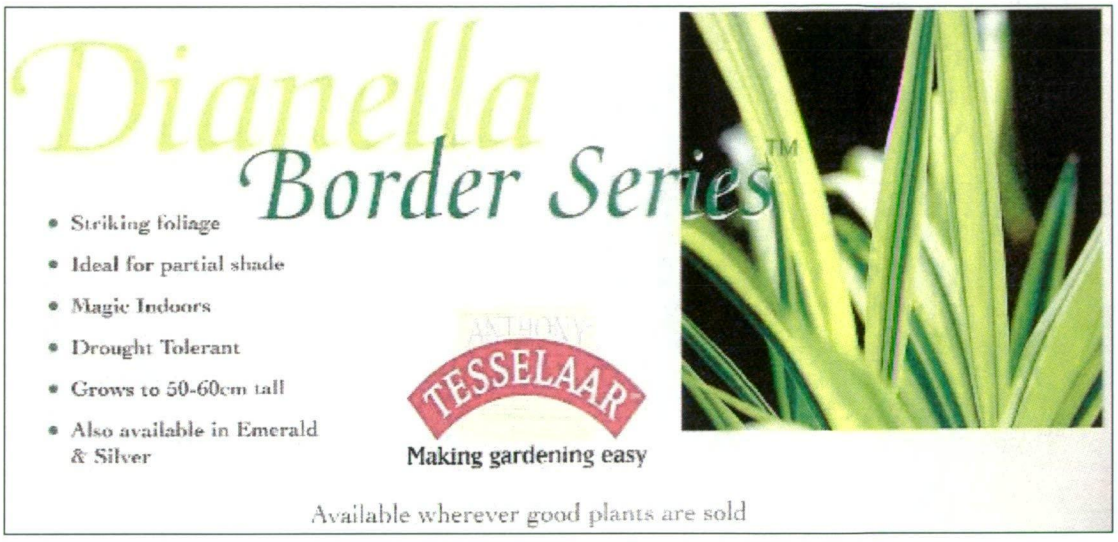

Plate 1.3. Advertisement for the "Border" series™ of *D. ensifolia* by Anthony Tesselaar International. From Gardening Australia Magazine, March 2002.

1.1.3 *Milligania*

The *Milligania* genus is endemic to Tasmania and comprises 5 species: *M. densiflora*, *M. longifolia*, *M. lindoniana*, *M. stylosa* and *M. johnstonii* (Williams, 1987; Collier, 1991; Elliot and Jones, 1993; Curtis and Morris, 1994). All occur above an altitude of 700 m and are often subject to extremely low temperatures (Elliot and Jones, 1993); as a result they are very frost-hardy plants (Wrigley and Fagg, 1996). The genus is named after Joseph Milligan (1807-1883), an English surgeon

and naturalist who worked in Tasmania from 1829-1860 (Williams, 1987; Elliot and Jones, 1993).

All species are very attractive and highly desirable for cultivation but are currently not often grown in Australian gardens (Elliot and Jones, 1993). *M. lindoniana* has been suggested as highly desirable, but probably not well suited to cultivation (Collier, 1991). However, other authors believe that Milliganias, in particular, *M. densiflora* (Wrigley and Fagg, 1996), have excellent potential for cool temperate regions (Elliot and Jones, 1993). The flowers are attractive, but they are also recommended for their foliage (Wrigley and Fagg, 1996).

Milliganias grow best in well drained soils with some organic matter (Elliot and Jones, 1993; Wrigley and Fagg, 1996). Elliot and Jones (1993) believe that planting in an open, but not hot, sunny site would be most successful. However, Wrigley and Fagg (1996) state that some shade is required. They also need plenty of moisture, especially in summer (Elliot and Jones, 1993; Wrigley and Fagg, 1996). As well as their use as a garden plant, where they are best suited to rockeries (Wrigley and Fagg, 1996), some species also grow well in containers (Elliot and Jones, 1993) (Plate 1.4).

Plate 1.4. *Milligania densiflora* pot plant specimen. Pot diameter = 20 cm.

Milligania can be propagated by seed or by division, however the evidence of propagation by seed is scarce as it is rarely, if ever, offered for sale. Division of the rhizome is usually successful provided that growth points are present, divisions are not too small and a moist potting medium is maintained at all times (Elliot and Jones, 1993).

1.1.4 *Diplarrena*

The *Diplarrena* genus consists of two species which are endemic in Eastern Australia (Blombery, 1979; Elliot and Jones, 1984; Cooke, 1986; Conn, 1994; Curtis and Morris, 1994; Barnard *et al.*, 2004). Both species occur in Tasmania, with one being endemic to the state (Curtis and Morris, 1994). The name *Diplarrena* is from the Greek "diploos" (double) and "arren" (male) which refers to the two fertile stamens (Elliot and Jones, 1984; Cooke, 1986). *Diplarrena moraea*, commonly known as the "Butterfly Flag" (Rotherham *et al.*, 1975; Elliot and Jones, 1984; Conabere and Garnet, 1987; Greig, 1993; Barnard *et al.*, 2004), the "White Iris" (Elliot and Jones, 1984; Collier, 1990; Greig, 1993; Conn, 1994) or the "White Flag Iris" (Rodway, 1922; Cameron, 1992; Curtis and Morris, 1994) is so named as it is similar to the South African genus *Moraea* (Rodway, 1922; Elliot and Jones, 1984). While *Diplarrena latifolia*, the "Western Flag Iris" (Kirkpatrick, 1997) is given this specific name due to the broadness of the leaves (Elliot and Jones, 1984).

Both species are showy and excellent for cultivation (Elliot and Jones, 1984). They are hardy and moderately frost tolerant (Page and Olds, 2004), especially *D. latifolia* , which will survive heavy frosts and even snow (Elliot and Jones, 1984). Due to their clumping nature they are ideal when mass planted as a border (Greig, 1993), in rock gardens or beside a water feature (Greig, 1993; Wrigley and Fagg, 1996; Barnard *et al.*, 2004). They are also excellent container plants (Blombery, 1972; Elliot and Jones, 1984; Greig, 1993). Individual flowers only last for 1 day, but they emerge in succession, so that the flowering period lasts for many weeks (Blombery, 1972; Elliot and Jones, 1984; Cameron, 1992; Greig, 1993). The foliage also provides variety and form within the garden (Elliot and Jones, 1984).

Diplarrena can be propagated by seed or division of the rhizome (Blombery, 1979; Elliot and Jones, 1984; Collier, 1990; Greig, 1993; Wrigley and Fagg, 1996; Barnard *et al.*, 2004; Page and Olds, 2004). According to Blombery (1979) small aerial plants develop on the stems of older plants and these can also be used for propagation purposes, simply by removing them from the parent plant and replanting. *Diplarrena* grows best in a protected position of the garden with ample moisture (Blombery, 1972; Greig, 1993; Page and Olds, 2004) but soils must be relatively well drained (Elliot and Jones, 1984; Greig, 1993; Wrigley and Fagg, 1996; Barnard *et al.*, 2004; Page and Olds, 2004). They will grow in areas of dappled shade or partial to full sun (Elliot and Jones, 1984; Barnard *et al.*, 2004; Page and Olds, 2004), but for *D. latifolia*, plants must receive lots of sun to flower at their best (Elliot and Jones, 1984; Wrigley and Fagg, 1996).

Selections of *D. latifolia* such as "Amethyst Fairy" are relatively popular in Australia and are also available in Europe (Elliot and Jones, 1984). Within Tasmania, *D. moraea* is often used by local councils as a landscaping plant, especially by roadsides.

1.1.5 *Isophysis*

Isophysis is a monotypic genus endemic to Tasmania (Cooke, 1986; Cameron, 1992; Toelken, 1993; Curtis and Morris, 1994; Kirkpatrick, 1997). The generic name *Isophysis* comes from the Greek "isos" (equal) and "physis" (growth), which refers to the six similar tepals (Cooke, 1986). The single species within this genus, *Isophysis tasmanica*, commonly known as "Hewardia" (Cooke, 1986; Collier, 1991; Toelken, 1993; Curtis and Morris, 1994; Wrigley and Fagg, 1996) is an unusual member of the Iridaceae family due to its superior ovary (Dahlgren *et al.*, 1985). However, the vegetative characteristics clearly show that it is a member of this family, and not the Liliaceae. It has been suggested that *Isophysis* resembles the ancestral Iridaceae species (Dahlgren *et al.*, 1985).

Due to the large size and unusual colour of the flower, *I. tasmanica* is highly desirable for horticultural purposes. However, at present it appears that these

characteristics have largely been ignored, and this is perhaps largely due to the species endemicity to Tasmania, its restricted distribution within the state and the fact that it is difficult to grow (Collier, 1991; Wrigley and Fagg, 1996). Propagation by seed is reportedly successful (Wrigley and Fagg, 1996), but efforts at division of the rhizome by the Australian National Botanic Gardens (ANBG) were unsuccessful. Plants grew well and flowered in pots at the ANBG but would not set seed despite attempts at pollination (Wrigley and Fagg, 1996). If these difficulties with propagation and cultivation can be overcome, *I. tasmanica* would be excellent as a rockery plant (Wrigley and Fagg, 1996) or associated with a water feature. A sunny well-drained site would probably be required (Wrigley and Fagg, 1996).

1.2 Botanical Description of the Liliaceae Family

Note: For the purposes of this thesis the treatment of the Liliaceae family is in conformity with that of the "Flora of Australia" Volume 15 (1987) and the "Students Flora of Tasmania" Part 4B (1994), which follows the concept of Cronquist, "An Integrated System of Classification of Flowering Plants" (1981). This treatment deals with the family in the broadest sense, incorporating genera that have been placed in a number of separate families by some authors (Curtis and Morris, 1994).

Plants in the Liliaceae family are perennial herbs (George, 1987; Stanley and Ross, 1989; Jessop, 1993; Conran, 1994; Curtis and Morris, 1994) or rarely annuals (Stanley and Ross, 1989; Curtis and Morris, 1994), shrubs or climbers (George, 1987; Stanley and Ross, 1989; Conran, 1994). The aerial shoots are annual or evergreen (George, 1987; Stanley and Ross, 1989; Conran, 1994) arising from rhizomes, bulbs, corms or tubers (George, 1987; Stanley and Ross, 1989; Jessop, 1993; Curtis and Morris, 1994). Roots are fleshy (Curtis and Morris, 1994) and fibrous or tuberous (George, 1987; Conran, 1994; Curtis and Morris, 1994). The leaves are simple (Conran, 1994; Curtis and Morris, 1994), alternate (George, 1987; Stanley and Ross, 1989; Conran, 1994; Curtis and Morris, 1994) or verticillate (Conran, 1994; Curtis and Morris, 1994) or rarely (not in Australia) opposite (George, 1987; Stanley and

Ross, 1989; Conran, 1994; Curtis and Morris, 1994), often all basal (George, 1987; Curtis and Morris, 1994), linear, lanceolate or ovate (George, 1987; Curtis and Morris, 1994) usually with parallel venation (Conran, 1994; Curtis and Morris, 1994), sessile (often sheathing at the base) or pseudopetiolate (George, 1987; Conran, 1994), sometimes resupinate (George, 1987), sometimes reduced to scales (George, 1987; Stanley and Ross, 1989; Conran, 1994; Curtis and Morris, 1994). Stems are simple or branched, leafy, bracteate or naked (Curtis and Morris, 1994), sometimes with branches reduced to cladophylls (George, 1987; Stanley and Ross, 1989; Conran, 1994; Curtis and Morris, 1994). Flowers are unisexual or bisexual (George, 1987; Conran, 1994; Curtis and Morris, 1994), actinomorphic or zygomorphic, in terminal or axillary spikes, racemes, corymbs, panicles or umbels, or sometimes solitary and terminal or axillary (George, 1987; Stanley and Ross, 1989; Conran, 1994; Curtis and Morris, 1994). There are 6 perianth segments in 2 similar or dissimilar whorls of 3, occasionally more or fewer, free or fused (George, 1987; Stanley and Ross, 1989; Jessop, 1993; Conran, 1994; Curtis and Morris, 1994), if fused a corona is sometimes present (George, 1987; Conran, 1994; Curtis and Morris, 1994). There are 3 or 6 stamens, or rarely 4 or 10-14 (George, 1987; Curtis and Morris, 1994) or (not in Australia) 8 or 12 (George, 1987). If less than 6 there may be staminodes present (George, 1987; Curtis and Morris, 1994). The stamens are either free or partially fused to the perianth or to one another (Jessop, 1993; Conran, 1994). The anthers are bilocular, dehiscing by longitudinal slits or apical pores (George, 1987; Stanley and Ross, 1989; Conran, 1994; Curtis and Morris, 1994). The dehiscence introrse, extrorse or latrorse (George, 1987; Curtis and Morris, 1994). The ovary is superior (George, 1987; Stanley and Ross, 1989; Jessop, 1993; Conran, 1994; Curtis and Morris, 1994), inferior or partially inferior (George, 1987; Conran, 1994; Curtis and Morris, 1994). There are usually 1 (George, 1987; Stanley and Ross, 1989; Conran, 1994) or 3 locules (George, 1987; Stanley and Ross, 1989; Jessop, 1993; Conran, 1994; Curtis and Morris, 1994) or rarely 4-7 (George, 1987; Stanley and Ross, 1989; Curtis and Morris, 1994); 1 to many ovules per loculus (George, 1987; Stanley and Ross, 1989; Jessop, 1993; Conran, 1994; Curtis and Morris, 1994) with basal, axile or parietal placentation (George, 1987; Curtis and Morris, 1994). There are 1 or 3 styles

(George, 1987; Stanley and Ross, 1989; Conran, 1994; Curtis and Morris, 1994), stigmas capitate, 2-3-lobed (George, 1987; Stanley and Ross, 1989; Curtis and Morris, 1994) or punctiform (Curtis and Morris, 1994), trifid or very small (George, 1987) or stigmas 3-7 (George, 1987; Stanley and Ross, 1989; Curtis and Morris, 1994). The fruit is a berry, capsule or dry and indehiscent (George, 1987; Stanley and Ross, 1989; Conran, 1994; Curtis and Morris, 1994). Seeds are globular to flattened (Conran, 1994), smooth or variously ornamented, sometimes winged (George, 1987; Conran, 1994; Curtis and Morris, 1994), rarely papillose or hairy (George, 1987; Curtis and Morris, 1994), aril (Curtis and Morris, 1994) or elaiosome sometimes present (George, 1987; Curtis and Morris, 1994). The endosperm lacks starch (George, 1987).

The Liliaceae is a large family of about 280 genera and 4000 species (George, 1987; Stanley and Ross, 1989; Conran, 1994; Curtis and Morris, 1994), or 180 genera and 3500 species worldwide (Jessop, 1993). It is widely distributed in both hemispheres (Jessop, 1993) in tropical to temperate regions (George, 1987; Stanley and Ross, 1989; Curtis and Morris, 1994). Many of the exotic members of the Liliaceae are common garden plants (Curtis and Morris, 1994) and cut flowers. The horticulturally important genera include: *Tulipa*, *Lilium*, *Muscari*, *Ornithogalum* and *Hyacinthus* (Jessop, 1993).

In Australia the number of genera and species varies according to author: 66 genera and 266 species (George, 1987; Conran, 1994), 57 genera and 241 species (Stanley and Ross, 1989), or 41 genera and 200 species (Jessop, 1993); of which 30 genera and 202 species are endemic and 46 species are naturalised (George, 1987). In Tasmania there are 26 genera, of which 20 are native to the state, the rest being introduced ornamentals that have become naturalised (Curtis and Morris, 1994). The Tasmanian Liliaceae genera and the alternative families they have been placed in by Dahlgren *et al.* (1985) in the "Families of the Monocotyledons" are shown in Table 1.1.

Table 1.1. Tasmanian Liliaceae genera and their alternative family placings according to Dahlgren *et al.* (1985). From: Curtis and Morris (1994).
* Introduced to Tasmania.

Genus	Family
Drymophila	Luzuriagaceae
Asparagus *	Asparagaceae
Astelia *Milligania*	Asteliaceae
Blandfordia	Blandfordiaceae
Hypoxis	Hypoxidaceae
Dianella *Thelionema*	Phormiaceae
Asphodelus * *Bulbine* *Herpolirion*	Asphodeliaceae
Arthropodium *Caesia* *Chamaescilla* *Dichopogon* *Laxmannia* *Sowerbaea* *Thysanotus* *Tricoryne*	Anthericaceae
Scilla *	Hyacinthaceae
Allium * *Nothoscordum* *	Alliaceae
Narcissus *	Amaryllidaceae
Campynema	Campynemaceae
Burchardia *Wurmbea*	Colchicaceae

Three native Tasmanian Liliaceae genera: *Blandfordia*, *Dianella* and *Milligania*, have been included in the present study and will be described further.

1.2.1 Botanical Description of the Liliaceae genera included in this study

1.2.1.1 *Blandfordia*

Blandfordia plants are tufted herbaceous perennials (Henderson, 1987a; Lamont *et al.*, 1990; Curtis and Morris, 1994; Johnson, 1996a; Greig, 1999). The storage organ has been variously described as a tuberous corm (Henderson, 1987a; Stanley and Ross, 1989; Curtis and Morris, 1994), a bulbous growth (Harris, 1949) or a rhizome (Dahlgren *et al.*, 1985; Knees, 1986), but it is actually a rhizomatous corm (Johnson, 1996a). The roots are fleshy-fibrous (Henderson, 1987a; Stanley and Ross, 1989; Curtis and Morris, 1994) or tuberous (Henderson, 1987a; Curtis and Morris, 1994). The linear leaves are acute and narrowly sheathing at the base (Henderson, 1987a; Cameron, 1992; Curtis and Morris, 1994), they are mostly crowded at the base of the stem (Collier, 1991; Johnson, 1996a), the margins are smooth or crenulate (Cochrane *et al.*, 1980; Cameron, 1992; Johnson, 1996a; Kirkpatrick, 1997; Greig, 1999). The stem has leaf-like bracts (Henderson, 1987a; Curtis and Morris, 1994). The inflorescence is a terminal raceme (Blombery, 1979; Cochrane *et al.*, 1980; Henderson, 1987a; Stanley and Ross, 1989; Greig, 1990, 1999; Cameron, 1992; Curtis and Morris, 1994) borne on a long, erect scape (Blombery, 1972, 1979; Cochrane *et al.*, 1980; Stanley and Ross, 1989; Lamont *et al.*, 1990). Individual flowers are bisexual, pedicellate, pendulous, each subtended at the base of the pedicel by a bract and a smaller bracteole (Henderson, 1987a; Curtis and Morris, 1994; Johnson, 1996a), they are actinomorphic (Henderson, 1987a; Curtis and Morris, 1994), gamopetalous (Curtis and Morris, 1994) and tubular to campanulate in shape (Henderson, 1987a; Stanley and Ross, 1989; Curtis and Morris, 1994; Johnson, 1996a). The perianth is petaloid with the limb being 6-lobed (Henderson, 1987a; Curtis and Morris, 1994) and the colour ranging from red to orange and pure yellow (Blombery, 1972, 1979; Henderson, 1987a; Stanley and Ross, 1989; Greig, 1990, 1993, 1999; Lamont *et al.*, 1990; Cameron, 1992; Curtis and Morris, 1994; Wrigley and Fagg, 1996; Kirkpatrick, 1997; Stewart, 2002). There are 6 stamens, the filaments are adnate to the perianth tube (Blombery, 1979; Henderson, 1987a; Curtis

and Morris, 1994), anthers are linear (Henderson, 1987a), versatile, included or slightly exerted, the dehiscence being latrorse (Henderson, 1987a; Curtis and Morris, 1994), by slits (Henderson, 1987a). The superior ovary is stipitate and 3 locular, the ovules in 2 rows per locule (Henderson, 1987a; Curtis and Morris, 1994), and numerous (Henderson, 1987a); the placentation is axile. The style is linear, the stigma capitate but 3-lobed (Henderson, 1987a; Curtis and Morris, 1994). The fruit is a capsule (Blombery, 1979; Henderson, 1987a; Collier, 1991; Stanley and Ross, 1989; Cameron, 1992; Curtis and Morris, 1994; Johnson, 1996a; Greig, 1999) that is stipitate with septicidal dehiscence (Henderson, 1987a; Curtis and Morris, 1994); it is erect, ± fusiform and prominently 3-angled (Henderson, 1987a). Seeds are numerous, linear (Henderson, 1987a; Curtis and Morris, 1994), brown (Blombery, 1979; Henderson, 1987a; Curtis and Morris, 1994; Greig, 1999) and papillose-hairy (Henderson, 1987a; Stanley and Ross, 1989; Curtis and Morris, 1994; Johnson, 1996a).

1.2.1.2 *Dianella*

Plants within the *Dianella* genus are herbaceous to shrub-like, rhizomatous perennials with fibrous, fleshy-fibrous or tuberous roots (Henderson, 1987b; Stanley and Ross, 1989; Wilson, 1993; Conran, 1994; Curtis and Morris, 1994). The true aerial stems are elongated or very short (Henderson, 1987b; Stanley and Ross, 1989; Wilson, 1993), short in Tasmanian species (Curtis and Morris, 1994). The lower leaves are often reduced to scale-like sheaths, the blades are linear or linear-elliptic, with the margins and midrib on the abaxial surface smooth or scabrous (Curtis and Morris, 1994). The adaxial surfaces of the upper sheath are variously occluded (fused) (Curtis, 1952; Wilson, 1993). Leaf-sheaths are open or closed, compressed laterally, and often keeled (Elliot and Jones, 1984). The inflorescence is described as compound-cymose and bracteate at least at the lower nodes (Henderson, 1987b; Stanley and Ross, 1989; Curtis and Morris, 1994). The flowers are bisexual, pedicellate (Henderson, 1987b; Stanley and Ross, 1989; Curtis and Morris, 1994) and actinomorphic (Curtis and Morris, 1994), in condensed or expanded raceme-like cymules (bostryces) (Henderson, 1987b; Stanley and Ross, 1989). The perianth is

petaloid, comprised of 6 segments in 2 subequal whorls, free, blue, purple or white in colour (Plate 1.5), and withering and persistent after anthesis (Hurst, 1942; Elliot and Jones, 1984; Henderson, 1987b; Wilson, 1993; Curtis and Morris, 1994).

Plate 1.5. Flowers of, clockwise from top left: *D. amoena*, *D. atraxis*, *D. caerulea* (unknown) variety and *D. caerulea* Hadea section, showing the wide range of flower colours in the genus. Scale bars = 3 mm.

There are 6 stamens and the filaments are thickened apically near the attachment point of the anther to form a globose or ovoid structure covered with nectar-secreting papillae (Dahlgren *et al.*, 1985; Wilson, 1993) (Plate 1.6). The anthers are erect (Hurst, 1942), basifixed (Conran, 1994) and dehisce extrorsely by pores which become slits (Hurst, 1942; Dahlgren *et al.*, 1985; Henderson, 1987b; Wilson, 1993; Conran, 1994; Curtis and Morris, 1994). The ovary is globose, superior (Henderson, 1987b; Curtis and Morris, 1994), usually 3-locular (Henderson, 1987b; Stanley and

Ross, 1989; Conran, 1994; Curtis and Morris, 1994); ovules are biseriate, 2-12 per locule, and pendulous. The placentation is axile (Henderson, 1987b; Curtis and Morris, 1994); the style is filiform; and the stigma is capitate, and minute (Henderson, 1987b; Stanley and Ross, 1989; Conran, 1994; Curtis and Morris, 1994). Fruit is a blue berry (occasionally white) which is depressed-globular to oblong in shape (Henderson, 1987b; Stanley and Ross, 1989; Curtis and Morris, 1994). The seed is obliquely obovate, usually transversely biconvex (Henderson, 1987b; Curtis and Morris, 1994); the testa is ± smooth or minutely sculptured (Henderson, 1987b), brown or black, shiny, rarely dull (Elliot and Jones, 1984; Stanley and Ross, 1989; Henderson, 1987b; Wilson, 1993; Conran, 1994; Curtis and Morris, 1994).

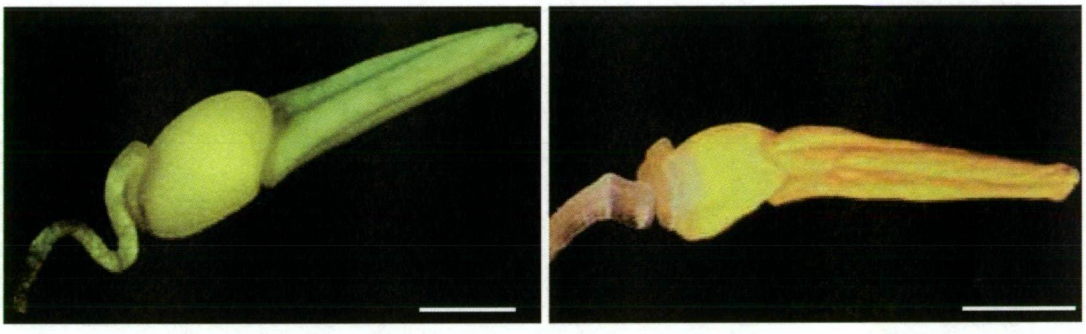

Plate 1.6. *Dianella porracea* (left) and *D. atraxis* (right) stamens, showing the apical thickenings of the filaments (near the attachment point of the anther) forming a globose or ovoid structure. Scale bars = 1mm.

1.2.1.3 *Milligania*

The genus *Milligania* contains plants which are tufted rhizomatous perennial herbs (Williams, 1987; Elliot and Jones, 1993; Curtis and Morris, 1994), the bases of which are clothed in the fibrous remains of leaf-sheaths from previous years (Curtis and Morris, 1994). The leafy stems are short and usually hidden (Williams, 1987; Elliot and Jones, 1993). Young leaves and the inflorescence have an indumentum of simple, dendroid or crisped hairs (Williams, 1987; Elliot and Jones, 1993; Curtis and Morris, 1994). Leaves are 3-ranked, linear to lanceolate, keeled, with sheaths closed at the base. Inflorescence is a bracteate, terminal panicle or raceme, sometimes much reduced; branches are subtended by foliaceous or membranous spathes, and bracts are

membranous (Williams, 1987; Elliot and Jones, 1993; Curtis and Morris, 1994). Flowers are bisexual, actinomorphic and pedicellate (Williams, 1987; Curtis and Morris, 1994). There are 6 perianth segments (Williams, 1987; Elliot and Jones, 1993; Curtis and Morris, 1994) united into a tube at the base (Williams, 1987; Curtis and Morris, 1994). The 6 stamens are inserted at the throat and dehisce introrsely by longitudinal slits. The 3-locular ovary is superior or basally adnate to the perianth tube, placentation axile; style 3-branched or styles 3, stigmas 3 (Williams, 1987; Curtis and Morris, 1994), stigmas are punctiform (Curtis and Morris, 1994). Fruit is a capsule, opening in 3 apical valves (Dahlgren *et al.*, 1985; Williams, 1987; Elliot and Jones, 1993). Seeds are several, being ovate to oblong (Curtis and Morris, 1994), with a black, glossy, hard testa (Williams, 1987).

1.2.2 Botanical Descriptions of the Liliaceae Species Included in this Study

One *Blandfordia* species, three *Dianella* species and one *Milligania* species feature in this study. The botanical description of each of these species follows.

1.2.2.1 *Blandfordia punicea* (Tasmanian Christmas Bell)

Leaves to approximately 35 cm (Henderson, 1987a), 15-45 cm (Cochrane *et al.*, 1980; Cameron, 1992), 1 m (Johnson, 1996a; Wrigley and Fagg, 1996; Kirkpatrick, 1997; Greig, 1999) or up to 1.5 m long (Curtis and Morris, 1994) and 4 (Henderson, 1987a) -12 mm (Henderson, 1987a; Curtis and Morris, 1994; Barnard *et al.*, 2004) or 1 (Greig, 1999) or 2 cm wide (Wrigley and Fagg, 1996). They are narrow-linear (Cameron, 1992; Greig, 1999), thick and tough (Cameron, 1992; Kirkpatrick, 1997), the laminar is ribbed or channelled (Henderson, 1987a; Cameron, 1992; Curtis and Morris, 1994; Wrigley and Fagg, 1996), the midrib is pale and prominent on the abaxial surface (Curtis and Morris, 1994; Kirkpatrick, 1997), the margins are crenulate (Cochrane *et al.*, 1980; Henderson, 1987a; Cameron, 1992; Curtis and Morris, 1994; Kirkpatrick, 1997; Greig, 1999; Barnard *et al.*, 2004) and recurved (Cameron, 1992; Johnson, 1996a; Wrigley and Fagg, 1996; Greig, 1999). Leaves are

dark green (Johnson, 1996a) or often reddish (Kirkpatrick, 1997) or purplish (Collier, 1991). The scape is stout and erect (Cameron, 1992; Curtis and Morris, 1994; Johnson, 1996a; Wrigley and Fagg, 1996; Greig, 1999), 30 cm (Cameron, 1992; Johnson, 1996a) to 1 m long (Henderson, 1987a; Cameron, 1992; Johnson, 1996a; Wrigley and Fagg, 1996; Greig, 1999) or up to 1.2 m high and up to 8 mm in diameter (Curtis and Morris, 1994), lower bracts are long and leaf-like, while upper bracts are progressively shorter and often wider than the leaves (Curtis and Morris, 1994). The inflorescence is a terminal raceme (Cochrane *et al.*, 1980; Henderson, 1987a; Cameron, 1992; Curtis and Morris, 1994; Johnson, 1996a) bearing between 15 and 50 individual flowers (Henderson, 1987a; Curtis and Morris, 1994; Kirkpatrick, 1997); the peduncle is up to 6 mm in diameter (Henderson, 1987a), the pedicels usually 2-4 cm long (Curtis and Morris, 1994) or 3-5 cm long (Cochrane *et al.*, 1980) at flowering, elongating and up to 6.5 cm long (Henderson, 1987a; Curtis and Morris, 1994) as the capsule matures. The corolla tube is usually 3.5-4.5 cm long (Henderson, 1987a; Curtis and Morris, 1994) or about 4 (Cochrane *et al.*, 1980; Greig, 1999) or 5 cm long (Collier, 1991; Johnson, 1996a) and red-brown (Henderson, 1987a), orange-red (Curtis and Morris, 1994; Kirkpatrick, 1997), scarlet (Collier, 1991; Johnson, 1996a) or occasionally yellow (Cameron, 1992; Curtis and Morris, 1994; Kirkpatrick, 1997) externally, and yellow internally (Henderson, 1987a; Cameron, 1992; Curtis and Morris, 1994; Johnson, 1996a; Barnard *et al.*, 2004) (Plate 1.7), the perianth tube is narrowly conical in shape (Henderson, 1987a; Curtis and Morris, 1994) and 1.5-2 cm wide at the throat (Henderson, 1987a; Greig, 1999), the lobes are ovate-acute and up to 8.5 mm long (Henderson, 1987a; Curtis and Morris, 1994), the 3 inner lobes have yellow margins externally (Henderson, 1987a; Cameron, 1992; Curtis and Morris, 1994; Johnson, 1996a). The filaments are 0.8-1.1 cm long (Henderson, 1987a) and adherent to the perianth tube to above the mid-point (Henderson, 1987a; Curtis and Morris, 1994). Anthers are oblong, 3.5 (Henderson, 1987a) to 4 mm long (Henderson, 1987a; Curtis and Morris, 1994). At anthesis, the ovary is about 1.5 cm long on a 1 cm long stipe, style shorter than the stamens (Curtis and Morris, 1994). The capsule is 3.5 (Curtis and Morris, 1994) -5.5 cm long, the stipe elongating and up to 4 cm long (Henderson, 1987a; Curtis and Morris, 1994).

The shape of the capsule is oblong to oblanceolate and the perianth is persistent (Curtis and Morris, 1994), the whole is held erect on the elongated pedicel (Collier, 1991; Curtis and Morris, 1994).

Plate 1.7. Flower colour variants of *Blandfordia punicea*, from scarlet through to orange. Scale bars = 2.5 cm.

B. punicea is endemic to Tasmania (Conabere and Garnet, 1987; Henderson, 1987a; Collier, 1991; Cameron, 1992; Curtis and Morris, 1994; Gorst, 1996; Johnson, 1996a; Wrigley and Fagg, 1996; Kirkpatrick, 1997; Greig, 1999; Barnard *et al.*, 2004). It commonly occurs in the West and South West of the state (Collier, 1991; Cameron, 1992; Curtis and Morris, 1994; Johnson, 1996a) from sea level to approximately 1000 m (Curtis and Morris, 1994) and occasionally reaching 1300 m (Johnson, 1996a), it also occurs in the Central Highlands (Curtis and Morris, 1994), in the far South East at sea level and in the North West where it is restricted to the coastal area at Rocky Cape National Park (Johnson, 1996a). *B. punicea* occurs in damp sandy and acid heaths, moorlands and hillsides (Henderson, 1987a; Curtis and Morris, 1994; Gorst, 1996; Johnson, 1996a) usually in rocky places (Kirkpatrick, 1997) and often growing in cracks in quartzite (Johnson *et al.*, 1994). It flowers from mid-spring to late summer (October to March) (Conabere and Garnet, 1987; Henderson, 1987a; Cameron, 1992) and has also been observed flowering in autumn (Conabere and Garnet, 1987).

1.2.2.2 *Dianella tasmanica* (Tasman Flax Lily, Blueberry)

Tufted perennial plant to 1.5 m high (Willis *et al.*, 1975; Cochrane *et al.*, 1980; Curtis and Morris, 1994; Greig, 1999; Barnard *et al.*, 2004). Stems clustered or up to 15 cm apart (Henderson, 1987b; Curtis and Morris, 1994). Rhizomes thick, subterranean (Elliot and Jones, 1984), yellow; roots fibrous (Henderson, 1987b; Wilson, 1993; Curtis and Morris, 1994). Leaves to 95 cm (Henderson, 1987b; Wilson, 1993; Conran, 1994) or up to 1.2 m long, basal sheaths and buds red or purplish (Curtis and Morris, 1994) (Plate 1.8); sheaths conduplicate, the margins free at the base but fused below the blade (Henderson, 1987b; Wilson, 1993; Curtis and Morris, 1994), the fused portion narrow-elliptical in transverse section (Elliot and Jones, 1984; Curtis and Morris, 1994); blade approximately 15-30 mm wide (Cochrane *et al.*, 1980; Henderson, 1987b; Wilson, 1993; Conran, 1994; Curtis and Morris, 1994; Greig, 1999), the margins shallowly recurved, margins and midrib on the abaxial surface regularly scabrous (Conran, 1994; Curtis and Morris, 1994). Inflorescence shorter than or (usually) exceeding the foliage (Henderson, 1987b; Wilson, 1993; Conran, 1994; Curtis and Morris, 1994; Barnard *et al.*, 2004), narrow cylindrical to conical in outline (Henderson, 1987b). Inflorescence branches and pedicels stout or slender (Curtis and Morris, 1994); cymules contracting distally, 2-5 flowered (Henderson, 1987b; Wilson, 1993), pedicels 5-22 mm long, evenly rounded (Henderson, 1987b). Perianth segments narrowly ovate to elliptic (Henderson, 1987b), lavender to violet (Henderson, 1987b; Wilson, 1993) or blue (Willis *et al.*, 1975; Cochrane *et al.*, 1980; Elliot and Jones, 1984; Cameron, 1992; Conran, 1994; Greig, 1999); 7-11 mm long, outer segments 5-7-nerved, inner segments 5-nerved (Henderson, 1987b; Wilson, 1993; Curtis and Morris, 1994) (Plate 1.8). Filament swelling bright golden yellow (Henderson, 1987b; Wilson, 1993; Conran, 1994; Curtis and Morris, 1994), 2.3-3 mm (Henderson, 1987b; Wilson, 1993) or 2.5-4 mm long (Curtis and Morris, 1994); anthers 2.3 (Henderson, 1987b) or 2.5 (Curtis and Morris, 1994) to 3 mm long, pale yellowish-brown (Henderson, 1987b; Curtis and Morris, 1994). Filaments much longer than the anthers (Elliot and Jones, 1984; Conran, 1994). Ovules 6-12 per locule (Henderson, 1987b; Curtis and Morris, 1994). Berry elongated, 1.2-2.5 cm long (Henderson, 1987b; Wilson, 1993; Curtis and Morris, 1994), violet to blue in

colour (Willis *et al.*, 1975; Cochrane *et al.*, 1980; Elliot and Jones, 1984; Cameron, 1992; Greig, 1999; Barnard *et al.*, 2004). Seed 3-4 mm long (Henderson, 1987b; Conran, 1994), testa smooth but minutely areolate, black, shiny (Henderson, 1987b).

Plate 1.8. *D. tasmanica* plant from the Southern Tablelands, NSW (left) (scale bar = 5cm), and *D. tasmanica* flower (right). Flower diameter = 0.75 cm.

Dianella tasmanica occurs in Victoria, NSW and Tasmania (Willis *et al.*, 1975; Cochrane *et al.*, 1980; Elliot and Jones; 1984, Curtis and Morris, 1994; Barnard *et al.*, 2004; Page and Olds, 2004). Within Tasmania it is widespread throughout all regions (Curtis, 1952; Cameron, 1992; Collier, 1992; Curtis and Morris, 1994). It often occurs in damp situations (Cameron, 1992; Collier, 1992; Curtis and Morris, 1994) from sea level to about 820 m in coastal dunes, wet sclerophyll, rainforest and lowland shrubberies (Curtis and Morris, 1994). It flowers in late spring and early summer (Cameron, 1992; Collier, 1992; Barnard *et al.*, 2004; Page and Olds, 2004).

1.2.2.3 *D. revoluta var. revoluta* (Spreading Flax Lily, Black-anther Flax Lily)

Plants up to 80 cm high (Henderson, 1987b; Conran, 1994; Curtis and Morris, 1994), stems clustered or up to 30 cm apart (Henderson, 1987b; Curtis and Morris, 1994). Rhizomes are thick, subterranean and much-branched (Elliot and Jones, 1984); roots fibrous (Elliot and Jones, 1984; Henderson, 1987b; Stanley and Ross, 1989; Wilson, 1993; Curtis and Morris, 1994). Leaves 15-85 cm long (Henderson, 1987b; Stanley and Ross, 1989; Curtis and Morris, 1994) and 0.4-1.2 cm wide (Henderson, 1987b; Wilson, 1993; Conran, 1994), often glaucous especially abaxially (Henderson, 1987b; Wilson, 1993; Conran, 1994). Basal parts of leaves reduced to sheaths, sheaths folded, margins free at the base but fused below the blade (Henderson, 1987b; Wilson, 1993; Curtis and Morris, 1994), the fused portion narrow-elliptical in transverse section (Curtis and Morris, 1994). Sheaths are commonly reddish (Wilson, 1993; Conran, 1994). Leaf margins revolute (Elliot and Jones, 1984; Henderson, 1987b; Fairley and Moore, 1989; Greig, 1993, 1999; Wilson, 1993; Barnard *et al.*, 2004), the abaxial surface has raised contiguous nerves (papillae) (Henderson, 1987b; Stanley and Ross, 1989; Wilson, 1993; Curtis and Morris, 1994). The inflorescence wholly or mostly exceeds the leaves (Henderson, 1987b; Stanley and Ross, 1989; Wilson, 1993; Conran, 1994; Curtis and Morris, 1994), branches are ascending or divaricate (Henderson, 1987b; Conran, 1994; Curtis and Morris, 1994), cymules 2-9 flowered (Stanley and Ross, 1989; Wilson, 1993). Perianth segments mid to dark blue or violet (Henderson, 1987b; Stanley and Ross, 1989; Greig, 1990; Wilson, 1993; Conran, 1994; Curtis and Morris, 1994) (Plate 1.9a) or rarely white (Elliot and Jones, 1984; Curtis and Morris, 1994); outer segments 5.5-10 mm long, 5-7-nerved, inner segments 5-9.5 mm long (Henderson, 1987b; Wilson, 1993; Curtis and Morris, 1994), broader than the outer segments, 3-5-nerved (Curtis and Morris, 1994) or 5-nerved (Henderson, 1987b; Wilson, 1993). Filament swellings 0.5-2.25 mm (Curtis and Morris, 1994) or 0.6-2.3 mm long (Henderson, 1987b; Wilson, 1993), bright yellow; anthers 2.5-4.5 mm long (Henderson, 1987b; Stanley and Ross, 1989; Curtis and Morris, 1994), pale brown to black (Elliot and Jones, 1984; Henderson, 1987b; Fairley and Moore, 1989; Stanley and Ross, 1989; Wilson, 1993; Conran, 1994;

Curtis and Morris, 1994; Greig, 1999). Ovules 4-10 per locule (Henderson, 1987b; Wilson, 1993; Curtis and Morris, 1994). The berry is ± globular (Conran, 1994; Curtis and Morris, 1994), 4-10 mm long (Henderson, 1987b; Stanley and Ross, 1989; Wilson, 1993; Conran, 1994; Curtis and Morris, 1994), blue or rarely white (Curtis and Morris, 1994) (Plate 1.9b). The shiny, black seeds are 2.5-5.2 mm long; the testa is minutely areolate-alveolate or colliculate, occasionally also irregularly grooved (Henderson, 1987b).

Plate 1.9. *D. revoluta* plant with blue flowers (a) (Scale bar = 0.5 cm), and white fruit (b) (Lens cap diameter = 6 cm).

D. revoluta var. *revoluta* occurs in all Australian states, except for the Northern Territory (Fairley and Moore, 1989; Greig, 1990, 1999; Wilson, 1993; Conran, 1994; Curtis and Morris, 1994). Within Tasmania it is widespread throughout all regions, from sea level to approximately 400 m. It occurs in open areas or under light eucalypt forest in damp to dry situations, in rocky to sandy soils (Curtis and Morris, 1994). It flowers in late spring and early summer (Elliot and Jones, 1984; Fairley and Moore, 1989; Collier, 1990; Greig, 1990, 1993, 1999; Barnard *et al.*, 2004).

1.2.2.4 *Dianella intermedia*

A tuft-forming plant which forms spreading patches 0.5-2.5 m wide and reaches a height of 0.5 m (Plate 1.10). Rhizomes slender, wiry and highly branched (Elliot and Jones, 1984). Leaves 10-50 cm long, 1-1.5 cm wide, distichous, linear, acuminate, the margins entire (*Ibid.*). Leaf colour varies from yellowish-green to dark green, leaves in a fan or tussock, a zone of partial fusion between the leaf-blade and sheath occurs (*Ibid.*). The leaf sheath laterally flattened and keeled. Inflorescence a panicle 20-40 cm long and loose; flowers approximately 1 cm in diameter, pale to dark blue, on stiffly arched pedicels (*Ibid.*). The filaments much shorter than the anthers. The berry approximately 1 cm long, pale to bright blue (*Ibid.*).

Dianella intermedia occurs on Norfolk Island and Lord Howe Island. It flowers during summer (Elliot and Jones, 1984).

Plate 1.10. *Dianella intermedia* plant. Scale bar = 5 cm.

1.2.2.5 *Milligania densiflora* (Common Milligania)

A perennial herb which reaches a height of 50 cm (Williams, 1987; Elliot and Jones, 1993). Leaves lanceolate or narrowly lanceolate, acuminate, erect to spreading (Williams, 1987; Elliot and Jones, 1993), up to 55 cm long and up to 5 cm wide (Curtis and Morris, 1994; Kirkpatrick, 1997), green or grey-green (Williams, 1987), often with a dense indumentum of long silky, simple hairs, the indumentum persistent or caducous, usually persistent on the midrib and margins (Curtis and Morris, 1994). Flowering stems usually 30-50 cm high (Curtis and Morris, 1994; Kirkpatrick, 1997), sometimes as low as 15 cm or as high as 75 cm; the panicle 15-33 cm (Williams, 1987; Elliot and Jones, 1993) or up to 40 cm long, dense and contracted or open with flowers ± crowded; the axis, branches and pedicels covered with a usually dense indumentum of long silky simple or dendroid hairs (Plate 1.11); bracts subtending the lower branches leaf-like, up to 35 cm long (Curtis and Morris, 1994).

Plate 1.11. Immature *Milligania densiflora* flower stem, showing the dense covering of hairs. Scale bar = 2 cm.

The panicle has 5-9 branches, the peduncle is 9-25 cm long, pedicels slender, 2-13 mm long (Williams, 1987). The perianth white, sometimes with a red tinge on the tube and throat, or very rarely, flowers pink (Curtis and Morris, 1994; Kirkpatrick, 1997). Flower tube 2-4 mm long, densely silky-hairy (Williams, 1987; Curtis and Morris, 1994), usually enclosing and basally adnate to the ovary (Williams, 1987); lobes erect to spreading, 6-10 mm (Curtis and Morris, 1994) or 12 mm long (Williams, 1987), pubescent along the centre externally (Williams, 1987; Curtis and Morris, 1994). Filaments broadly subulate, 0.2-1 mm long; anthers 0.6-0.75 mm long (Curtis and Morris, 1994). The ovary robust, 4 mm long, 3-lobed, style short and deeply trifid, or 3 styles (Williams, 1987). Capsule oblong, exerted (Williams, 1987), 3.5-5 mm long (Williams, 1987; Curtis and Morris, 1994). Seeds 1.75-2 mm long, navicular (Curtis and Morris, 1994).

Milligania densiflora is the most common and widespread of the Milliganias (Williams, 1987; Curtis and Morris, 1994). It, like the rest of the genus, is a Tasmanian endemic, and occurs in the Central Highlands, Mt Field, South West and West Coast at altitudes of 700 - 1500 m (Curtis and Morris, 1994). It is a dominant member of the alpine sedgeland community and is also found in heath, coniferous heath, bolster heath and deciduous heath (Kirkpatrick, 1997), usually in boggy or damp situations or on cliff ledges (Curtis and Morris, 1994) (Plate 1.12). *M. densiflora* flowers from November to December (Williams, 1987; Cameron, 1992) or sometimes as early as October (Elliot and Jones, 1993).

Plate 1.12. *Milligania densiflora* plant, Hartz Mountain, southern Tasmania. Scale bar = 2.5 cm.

1.3 Botanical Description of the Iridaceae Family

The majority of plants within the Iridaceae family are perennial herbs, but occasionally they are annuals or small shrubs (Cooke, 1986; Curtis and Morris, 1994). They may be evergreen or the leaves and flowers annual (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994). The storage organs are usually corms or rhizomes (Toelken, 1993; Conn, 1994) and occasionally bulbs (Dahlgren *et al.*, 1985; Cooke, 1986; Stanley and Ross, 1989; Curtis and Morris, 1994). Leaves are generally distichous with closed basal sheaths and parallel venation (Dahlgren *et al.*, 1985; Cooke, 1986; Conn, 1994; Curtis and Morris, 1994). They may be narrow or broad (Dahlgren *et al.*, 1985) are usually equitant and unifacial, flat, sometimes winged or terete and cruciform in section or bifacial and channelled (Cooke, 1986; Curtis and Morris, 1994). Leaves are glabrous or with simple hairs (Dahlgren *et al.*, 1985). The inflorescence is a spike, a panicle with spicate branches or of reduced cymes or a solitary terminal flower. If the inflorescence is spicate it comprises solitary flowers within a pair of spathe-bracts; if cymose the cluster of flowers is subtended by a pair of spathe-bracts and each flower is subtended by a bracteole (Curtis and Morris, 1994). The flowers are bisexual and actinomorphic or zygomorphic (Dahlgren *et al.*, 1985; Cooke, 1986; Stanley and Ross, 1989; Conn, 1994; Curtis and Morris, 1994) and are either sessile or pedicellate (Curtis and Morris, 1994). The perianth has 6 segments, 3 sepals and 3 petals in 2 similar or differentiated whorls (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994). Sometimes the inner whorl of petals is vestigial (Cooke, 1986; Conn, 1994). The perianth segments are free or basally connate forming a tube (Cooke, 1986; Stanley and Ross, 1989; Conn, 1994; Curtis and Morris, 1994). There are 3 stamens (2 in *Diplarrena)* which are inserted on the perianth tube opposite the sepals (Dahlgren *et al.*, 1985; Cooke, 1986; Stanley and Ross, 1989; Conn, 1994; Curtis and Morris, 1994). The filaments are narrow and may be free or partially connate (Dahlgren *et al.*, 1985; Stanley and Ross, 1989; Conn, 1994). Anthers are basifixed to ± versatile (Dahlgren *et al.*, 1985; Cooke, 1986; Conn, 1994), bilocular, and dehiscence is usually extrorse by longitudinal slits

(Dahlgren *et al.*, 1985; Cooke, 1986; Conn, 1994; Curtis and Morris, 1994). The gynoecium is comprised of 3 fused carpels (Cooke, 1986; Conn, 1994), the ovary is inferior (superior in *Isophysis*) and trilocular with axile placentation (Cooke, 1986; Stanley and Ross, 1989; Conn, 1994; Curtis and Morris, 1994) or rarely (not in Australia) unilocular with parietal placentation (Cooke, 1986; Stanley and Ross, 1989). The style is terminal with 3 branches or lobes, the branches are either entire or divided or petaloid; stigmas papillose (Dahlgren *et al.*, 1985; Cooke, 1986; Conn, 1994; Curtis and Morris, 1994). Each locule usually contains numerous anatropous ovules, or rarely few or even a single ovule (Dahlgren *et al.*, 1985; Cooke, 1986). The Iridaceae fruit is a capsule with a thin to leathery, or rarely hard, wall (Dahlgren *et al.*, 1985). It is loculicidally dehiscent from the apex, in 3-valves (Cooke, 1986; Stanley and Ross, 1989; Conn, 1994). The seeds are endospermic, sometimes arillate (Cooke, 1986; Conn, 1994) with shapes ranging from globose to ellipsoid or pyriform, angular to flattened, and they may be winged or unwinged (Dahlgren *et al.*, 1985; Curtis and Morris, 1994).

The Iridaceae family contains approximately 85 genera with 1500 species (Cooke, 1986; Stanley and Ross, 1989; Conn, 1994; Curtis and Morris, 1994). It is widely distributed throughout tropical and temperate regions of the world (Cooke, 1986; Stanley and Ross, 1989; Toelken, 1993; Conn, 1994; Curtis and Morris, 1994) with a major centre of diversity in southern Africa and secondary centres in South America and the eastern Mediterranean area (Dahlgren *et al.*, 1985; Cooke, 1986; Conn, 1994; Curtis and Morris, 1994). Many species and hybrids are important horticulturally in the cut flower trade and as garden ornamentals (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994) in particular those from the following genera: *Babiana*, *Dierama*, *Dietes*, *Freesia*, *Gladiolus*, *Iris*, *Ixia* and *Sparaxis* (Cooke, 1986; Conn, 1994).

In Australia there are 5 (Cooke, 1986) or 7 (Toelken, 1993) native genera, 4 of which occur in Tasmania (Curtis and Morris, 1994). Also in Tasmania there are 12 introduced genera (Curtis and Morris, 1994) which have become naturalised and some of which are now classified as weeds within the state (eg.*Watsonia* [Wind *et al.*, 2003]).

The native Tasmanian genera are: *Diplarrena*, *Isophysis*, *Libertia* and *Patersonia*. The first two genera have been included in the present study and will be described further.

1.3.1 Botanical Description of the Iridaceae genera included in this study

1.3.1.1 *Diplarrena*

Plants in the *Diplarrena* genus are evergreen perennial herbs which are densely tufted and rhizomatous (Elliot and Jones, 1984; Cooke, 1986; Conn, 1994; Curtis and Morris, 1994; Page and Olds, 2004) (Plate 1.13). Basal leaves are equitant, linear-ensiform, flat and glabrous, sheathing at the base, unifacial above, finely striate (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994) (Plate 1.13). The scape is erect, terete, simple or rarely branched with 2-4 reduced leaves (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994). The inflorescence is a cluster, or rhipidium (Cooke, 1986) of 3-6 (Cooke, 1986; Conn, 1994) or up to 12 flowers, which open successively (Blombery, 1972; Curtis and Morris, 1994) and are subtended by a pair of subopposite herbaceous spathe-bracts (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994).

Plate 1.13. *Diplarrena moraea* plant, showing short thickened rhizome, fibrous roots, and linear ensiform, flat, glabrous leaves.

The flowers are zygomorphic, pedicellate and separated by membranous bracteoles. The sepals and petals are free and clawed. Sepals are subequal, broad, spreading (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994) and white (Blombery, 1972; Curtis and Morris, 1994). The petals are oblong and shorter than the sepals (Cooke, 1986; Conn, 1994), the anterior pair are spreading (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994) and white with ± prominent purple nervation (Curtis and Morris, 1994), while the posterior segment is hooded over the stamens (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994). There are 2 fertile stamens, the 3rd lacking an anther (known as a staminode), the filaments free, flattened and unequal - the staminode is shorter (Blombery, 1979; Elliot and Jones, 1984; Cooke, 1986; Conn, 1994; Curtis and Morris, 1994). The anthers have a versatile attachment, are oblong in shape, unequal and oblique (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994). The cylindrical ovary is at first included within the spathe, the pedicel then elongates and becomes stouter as the capsule matures (Curtis and Morris, 1994). The style is filiform and simple (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994), exceeding the anthers (Cooke, 1986; Conn, 1994). The stigma is 3-lobed, with the lobes flattened and unequal (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994), papillose on the upper surface (Cooke, 1986). The fruit is a 3-angled capsule (Blombery, 1979; Elliot and Jones, 1984) which is exserted (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994). Seeds are numerous (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994), disciform, brown (Curtis and Morris, 1994); aril absent (Cooke, 1986; Conn, 1994).

1.3.1.2 *Isophysis*

Isophysis is a monotypic genus endemic to Tasmania (Cooke, 1986; Curtis and Morris, 1994). For further information see the species description of *Isophysis tasmanica* (Section 1.3.2.3).

1.3.2 Botanical Descriptions of the Iridaceae Species Included in this Study

Two *Diplarrena* species and the single *Isophysis* species feature in this study. The botanical description of each of these species follows.

1.3.2.1 *Diplarrena moraea* (White Flag Iris, Butterfly Flag, White Iris)

Leaves 10-70 (Cooke, 1986; Conn, 1994) or -75 cm long (Curtis and Morris, 1994) and 5-10 (Rotherham *et al.*, 1975; Cooke, 1986; Conn, 1994) or 2.5-9 mm wide (Curtis and Morris, 1994), dark green to slightly glaucous (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994). Scape 20 (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994) -100 (Cooke, 1986; Conn, 1994) or 105 cm long (Curtis and Morris, 1994), exceeding leaves (Cooke, 1986; Conn, 1994). Spathe-bracts subequal, linear-lanceolate (Cooke, 1986; Curtis and Morris, 1994), 4-8 (Cooke, 1986; Conn, 1994) or -8.5 cm long (Curtis and Morris, 1994), 6-12 mm wide (Cooke, 1986; Conn, 1994), green, glabrous (Cooke, 1986; Conn, 1994), prominently striate (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994). Flowers to 6 cm across, honey-scented (Elliot and Jones, 1984). Outer perianth segments oblanceolate-obovate (Curtis and Morris, 1994), tapering to the claw (Cooke, 1986; Curtis and Morris, 1994), entire, slightly concave, 2.5-3.5 cm long (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994), 1.3-2 (Curtis and Morris, 1994) or 1.5-2.2 cm wide (Cooke, 1986; Conn, 1994). Inner perianth segments white or ± purple-nerved, anterior segments oblong, obtuse or retuse, approximately 2 cm long (Curtis and Morris, 1994), often yellow towards the apex, purple nervation pale to prominent (Cooke, 1986; Conn, 1994; Curtis and Morris, 1994), margins white, posterior segment broad-oblanceolate, purple nervation ± prominent, fading towards the margins (Curtis and Morris, 1994). Fertile filaments 4-8 mm long (Cooke, 1986; Conn, 1994) or 5-9 mm long (Curtis and Morris, 1994); staminode approximately 2 mm (Curtis and Morris, 1994) or 2-4 mm long (Cooke, 1986; Conn, 1994); anthers 2-3 mm (Cooke, 1986; Conn, 1994) or 2.5-4 mm long (Curtis and Morris, 1994). The ovary is 1.5-2 cm long (Cooke, 1986; Conn, 1994;

Curtis and Morris, 1994); style is 0.8-1.2 cm (Curtis and Morris, 1994) or 1.2-1.5 cm long (Cooke, 1986; Conn, 1994); stigmatic lobes approximately 2.5 mm long (Curtis and Morris, 1994). Capsules are cylindrical to clavate, triquetrous, 2-2.5 cm long and approximately 7 mm wide (Cooke, 1986; Conn, 1994), rarely more than 4 maturing in each inflorescence (Curtis and Morris, 1994). Seeds are orbicular, brown and approximately 3 mm in diameter (Cooke, 1986: Conn, 1994).

Diplarrena moraea occurs in NSW, Victoria and Tasmania (Rotherham *et al.*, 1975; Blombery, 1979; Elliot and Jones, 1984; Cooke, 1986; Conabere and Garnet, 1987; Cameron, 1992; Conn, 1994; Curtis and Morris, 1994; Wrigley and Fagg, 1996; Barnard *et al.*, 2004; Page and Olds, 2004). It is widespread in Tasmania (Cooke, 1986) from sea level to about 1000 m, in a wide range of situations, from coastal dunes and scrub, heaths, rocky banks, under light forest cover and on the margins of wet sclerophyll forest. It can be found throughout the state in the Midlands, the North West, North East, Ben Lomond, East Coast, South West, West Coast and the Central Highlands (Curtis and Morris, 1994). *D. moraea* flowers from late spring to early summer (Collier, 1990; Cameron, 1992; Greig, 1993; Barnard *et al.*, 2004; Page and Olds, 2004) from September to December (Blombery, 1979; Conabere and Garnet, 1987) or October to January (Cooke, 1986; Conn, 1994).

1.3.2.2 *Diplarrena latifolia* (Western Flag Iris)

Leaves 25 (Curtis and Morris, 1994; Kirkpatrick, 1997) or 30 (Cooke, 1986) -100 cm long (Cooke, 1986; Curtis and Morris, 1994; Kirkpatrick, 1997) and 10-20 (Cooke, 1986) or -24 mm wide (Curtis and Morris, 1994; Kirkpatrick, 1997), usually dark green (Curtis and Morris, 1994), slightly glaucous (Cooke, 1986). Scape 50 (Curtis and Morris, 1994) or 60 (Cooke, 1986) -120 cm long (Cooke, 1986; Curtis and Morris, 1994), exceeding the leaves (Cooke, 1986; Kirkpatrick, 1997). Spathe-bracts linear-lanceolate, equal to unequal, 6-13 cm long (Curtis and Morris, 1994) or 6.5-8.5 cm long, 10-12 mm wide, green, glabrous (Cooke, 1986), prominently striate (Cooke, 1986; Curtis and Morris, 1994). Flowers to 6 cm across (Elliot and Jones, 1984). Outer perianth segments obovate to almost orbicular, 2.5 (Curtis and Morris, 1994) or 3.5 (Cooke, 1986) -4.2 cm long (Cooke, 1986; Curtis and Morris, 1994) and 1.5-3.7

(Curtis and Morris, 1994) or 2.5-3 cm wide (Cooke, 1986), abruptly contracted to the short claw, entire or emarginate, concave (Cooke, 1986; Curtis and Morris, 1994). The inner perianth segments 1.4-2.8 cm long, anterior segments ± prominently nerved, the colour more prominent at the margins (Curtis and Morris, 1994), often yellow toward the apex (Cooke, 1986; Curtis and Morris, 1994) or less often deep purple (Curtis and Morris, 1994); posterior segment oblanceolate-obovate, ± purple-nerved, the colour extending to the margins (Curtis and Morris, 1994) (Plate 1.14).

Plate 1.14. *Diplarrena latifolia* flower, showing purple nervation on the posterior inner perianth segments. Flower diameter = 5.5 cm.

The fertile filaments 4-8 mm (Cooke, 1986) or 11-15 mm long; the staminode approximately 2 mm long (Curtis and Morris, 1994) or 2-4 mm long (Cooke, 1986); and the anthers 3-3.5 mm long. The ovary is 10-20 mm long; the style 12-15 mm long; and the stigmatic lobes approximately 2 mm long (Curtis and Morris, 1994). The capsule is oblong, trigonous, 2.5 (Cooke, 1986; Curtis and Morris, 1994) -3 cm long (Curtis and Morris, 1994) and approximately 9 mm wide (Cooke, 1986), rarely more than 4 maturing in each inflorescence (Curtis and Morris, 1994). Seeds are orbicular, approximately 3 mm in diameter, brown (Cooke, 1986).

Diplarrena latifolia is endemic to Tasmania (Elliot and Jones, 1984; Cooke, 1986; Curtis and Morris, 1994; Wrigley and Fagg, 1996; Kirkpatrick, 1997; Barnard *et al.*, 2004; Page and Olds, 2004) and its distribution is restricted to the South West, West Coast (Cooke, 1986; Curtis and Morris, 1994) and Central Highlands (Curtis and Morris, 1994). It grows from near sea level to about 1000 m and favours damp conditions (Curtis and Morris, 1994). It mainly occurs in heathland communities, including peaty moorland near the coast (Cooke, 1986) and alpine heath and sedgeland (Kirkpatrick, 1997) (Plate 1.15). *D. latifolia* flowers in summer (Collier, 1990; Wrigley and Fagg, 1996; Page and Olds, 2004) from January to March (Cooke, 1986).

Plate 1.15. *Diplarrena latifolia* clump of plants in sub-alpine heathland, near Lake St Clair, Tasmania. Scale bar = 8 cm.

1.3.2.3 *Isophysis tasmanica* (Hewardia)

Isophysis tasmanica plants are tufted, glabrous, evergreen perennials (Cooke, 1986; Curtis and Morris, 1994). The storage organ is a rhizome (Dahlgren *et al.*, 1985; Cooke, 1986; Cameron, 1992; Curtis and Morris, 1994; Kirkpatrick, 1997) which is woody and persistent (Cooke, 1986; Cameron, 1992; Curtis and Morris, 1994). Leaves are numerous, basal, linear-ensiform (Cooke, 1986; Curtis and Morris, 1994), equitant (Cameron, 1992; Curtis and Morris, 1994) in fans (Collier, 1991; Cameron, 1992; Curtis and Morris, 1994), 5-30 cm long, 3-5 mm wide (Cooke, 1986; Curtis and Morris, 1994), glaucous (Cooke, 1986; Collier, 1991; Curtis and Morris, 1994), margins narrow, brown and scarious (Cooke, 1986; Curtis and Morris, 1994). The scape is erect, terete, unbranched, exceeding the leaves (Cooke, 1986; Curtis and Morris, 1994), 15-30 cm (Cameron, 1992) or up to 40 cm long (Curtis and Morris, 1994). It is smooth and bears 1 or 2 (Cooke, 1986) or 1-3 lanceolate reduced leaves which are 3-8 cm long (Cooke, 1986; Curtis and Morris, 1994) and a pair of subopposite spathe-bracts subtending the solitary terminal flower (Cameron, 1992; Curtis and Morris, 1994). Bracts are 2.5-4.5 (Cooke, 1986) or 2.5-6 cm long (Curtis and Morris, 1994) and 5-8 mm wide (Cooke, 1986; Curtis and Morris, 1994), pale brown or purplish (Curtis and Morris, 1994), caducous (Cooke, 1986). Flowers are pedicellate (Cooke, 1986; Curtis and Morris, 1994) and up to 6 (Wrigley and Fagg, 1996) or 8 cm in diameter (Cameron, 1992). Sepals and petals are equal, shortly clawed and united at the base forming a short floral tube (Cooke, 1986; Curtis and Morris, 1994), 2-4 cm (Cooke, 1986) or 2.5-6 cm long (Curtis and Morris, 1994) and 3-9 mm wide (Cooke, 1986; Curtis and Morris, 1994), dark purple (Kirkpatrick, 1997) or rarely yellow (Rodway, 1922; Collier, 1991; Cameron, 1992; Curtis and Morris, 1994; Wrigley and Fagg, 1996), buff (Curtis and Morris, 1994) or brownish-purple (Rodway, 1922; Curtis and Morris, 1994; Wrigley and Fagg, 1996) (Plate 1.16). The flower has 3 stamens (Cooke, 1986; Cameron, 1992; Curtis and Morris, 1994) which are spreading. The filaments are flattened, narrowly triangular, 4 (Cooke, 1986; Curtis and Morris, 1994) -5 mm (Cooke, 1986) or -7.5 mm long (Curtis and Morris, 1994); anthers are oblong, dorsifixed, yellow (Cooke, 1986; Curtis and Morris, 1994) or purple (Curtis and Morris, 1994), 4-6 mm (Cooke, 1986)

or 5-9 mm long (Curtis and Morris, 1994). The ovary is superior (Dahlgren *et al.*, 1985; Cooke, 1986; Curtis and Morris, 1994), conical, 4 (Cooke, 1986; Curtis and Morris, 1994) -6 mm (Cooke, 1986) or -7 mm long (Curtis and Morris, 1994); style approximately 3 mm long, simple, stigma with 3 recurved lobes, 1 (Cooke, 1986; Curtis and Morris, 1994) -2 mm (Cooke, 1986) or -2.5 mm long (Curtis and Morris, 1994).

Plate 1.16. *Isophysis tasmanica* flower (left), and clump of plants growing in sphagnum in a rocky crevice (right), at Clear Hill, South West Tasmania. Lens cap diameter = 6 cm.

The capsule is ovoid, prominently veined (Cooke, 1986; Curtis and Morris, 1994) acute and shiny (Cooke, 1986), 1-2 cm long (Cooke, 1986; Curtis and Morris, 1994), the pedicel elongates up to 6 cm long. The seeds are numerous, angular, approximately 2 mm long, light brown (Cooke, 1986; Curtis and Morris, 1994).

Isophysis tasmanica is endemic to Tasmania (Cooke, 1986; Cameron, 1992; Toelken, 1993; Curtis and Morris, 1994; Kirkpatrick, 1997). Its distribution is restricted to the Central Highlands (Curtis and Morris, 1994), South West and West Coast of the state, where it grows from sea level (Cooke, 1986; Cameron, 1992; Curtis and Morris, 1994) in wet peaty moorlands (Cooke, 1986; Cameron, 1992), up to altitudes of about 1300 m (Curtis and Morris, 1994) often on exposed mountain summits (Cooke,

1986). It occurs on gravel slopes, rock crevices, in grass and herbfield and alpine shrubberies (Curtis and Morris, 1994) (Plate 1.16). According to Kirkpatrick (1997) it is a dominant member of the alpine sedgeland community and a component of bolster heath, deciduous heath, coniferous heath and alpine heath. It flowers in summer (Collier, 1991) from December to January (Cooke, 1986; Cameron, 1992).

1.4 Objectives of the Study

The genera and species that have just been described are representative of many that possess highly desirable characteristics for commercial horticulture markets. However, in the case of some of these genera, especially *Milligania* and *Isophysis*, these characteristics have largely been ignored, most likely due to difficulties involved with their propagation. This project initiates an investigation of various botanical aspects of *Blandfordia*, *Diplarrena* and *Isophysis* and expands on previous research work undertaken on *Dianella* and *Milligania* (Sward, 1995). In most cases these native Tasmanian Liliaceae and Iridaceae genera have not been previously studied in great detail. The results will be used to assess the potential of the six genera for horticulture markets.

It should be noted that due to the previous work undertaken on the *Dianella* genus, in some areas of the present study this genus will occasionally be utilised more often than the others.

A wide range of botanical subject areas will be drawn from in order to achieve the objectives. Within the context of assessing the potential of these genera for commercial horticulture markets, the project comprised three specific aims:

1. To determine the natural germination behaviour of all species, and to test a number of methods which may enhance germination, thus determining the commercial viability of propagation by seed;

2. To develop micropropagation protocols for all species, so that rapid and reliable propagation methods are available; and

3. To further investigate the influence of environmental factors on floral initiation of *Dianella tasmanica*, and to determine whether this species can be induced to flower out-of-season.

Chapter 2
Seed Germination

2.1 Introduction

Propagation by seed is not a commonly used commercial method for production of native Liliaceae and Iridaceae. However, the use of seed is necessary in the development of new cultivars and for the maintenance of genetic diversity. Therefore, it is important to determine the natural germination rates of these species, to recognise any barriers to germination that may be present, and to determine methods that may enhance germination and overcome such barriers.

The majority of seeds from Australian native plants are soft and will germinate easily as long as the seed is viable (i.e. has gone through the necessary stages of development so that the seed is capable of germination) and is supplied with the necessary environmental conditions: oxygen, water, the correct temperature, light (or darkness) and the correct depth of sowing (Ralph, 1994; Stewart and Stewart, 1999; Stewart, 1999b). However, some species generally exhibit low or sporadic germination until they undergo some change or treatment. These seeds are in a state of dormancy. Dormancy is described as "a resting or quiescent condition with reduced metabolism" (Lawrence, 1989). It delays the germination of seeds for periods ranging from months to many years (Stewart and Stewart, 1999) until there are favourable conditions, thus aiding the long-term survival of the species (Hartmann and Kester, 1983; Ralph, 1994; Stewart, 1999b). Three major types of dormancy have been recognised: innate, induced and enforced (Robin, 1991).

Innate dormancy is defined as "a condition of seeds as they leave the parent plant which prevents germination because of some property of the seed" (Robin, 1991).

This type of dormancy may be controlled by a biochemical trigger, for example, moisture, light or temperature (Hartmann and Kester, 1983; Robin, 1991; Salisbury and Ross, 1992; Ralph, 1994), or by chemical or mechanical inhibitors.

Chemical germination inhibitors may be present within the seed itself, within the seed coat, or within the fruit that surrounds the seed (Khan, 1977; Hartmann and Kester, 1983; Robin, 1991; Ralph, 1994; Stewart, 1999b). Under natural conditions, seeds which contain inhibitory chemicals, such as *Billardiera* and *Correa*, will not germinate until there has been a long period of rainfall which leaches the chemicals from the seed (Ralph, 1994). Species in which the inhibitor is present within the fruit are generally those whose seeds are dispersed by birds or mammals (Robin, 1991). When the fruit passes through the animals gut the flesh is digested, thus removing the inhibitor (Robin, 1991).

Another form of chemical inhibition is found in seeds having "after-ripening" periods. When these seeds are shed from the parent plant they will not germinate (Black, 1972; Mayer and Poljakoff-Mayber, 1975; Wareing and Phillips, 1981; Bryant, 1985). Germination is delayed in these seeds until certain biochemical changes occur within them (Robin, 1991). Many native grasses and herbaceous species have an after-ripening period and won't germinate for 3 - 12 months after collection (Ralph, 1994). Such periods ensure that seeds will only germinate when it is an appropriate time of the year (Robin, 1991; Ralph, 1994). Therefore, after-ripening is a characteristic of species from areas with strongly seasonal climates (Robin, 1991).

Mechanical inhibitors to seed germination are caused by hard seedcoats (testa's), which prevent germination by restricting the entry of water or oxygen to the seed (Mayer and Poljakoff-Mayber, 1975; Ralph, 1994; Wrigley and Fagg, 1996; Stewart, 1999b; Adkins and Bellairs, 2000) and inhibit the expansion of the embryo during germination (Mayer and Poljakoff-Mayber, 1975; Hartmann and Kester, 1983; Robin, 1991; Bewley and Black, 1994).

Another form of innate dormancy is located within the embryo (Adkins and Bellairs, 2000). When this type of dormancy is present the embryo itself may be immature

and need to undergo further development before germination can occur, and/or the embryo may contain mechanisms that interfere with gene repression or activation, respiration processes or mobilisation and utilisation of food reserves (Adkins and Bellairs, 2000).

Induced dormancy is an "acquired condition caused by some experience after ripening" (Robin, 1991). Sometimes described as "secondary dormancy", dormancy may be induced if seeds are exposed to conditions that are unsuitable for germination (Bryant, 1985). For example, the failure to provide light to light-requiring seeds can induce dormancy (Mayer and Poljakoff-Mayber, 1975).

Enforced dormancy is defined as "the inability to germinate due to environmental restraints". For example, lack of water, low temperature or poor aeration (Robin, 1991).

2.1.1 Methods used to overcome Innate Seed Dormancy

For many species with innate seed dormancy an appropriate seed treatment can overcome the inability to germinate (Ralph, 1994; Wrigley and Fagg, 1996).

2.1.1.1 Physical or Mechanical Dormancy

The physical dormancy caused by hard seedcoats is the easiest type of dormancy to overcome (Wrigley and Fagg, 1996). The hard or thick seedcoats must be broken or pierced in some way to allow moisture and oxygen to penetrate the seed (Ralph, 1994; Wrigley and Fagg, 1996). Under natural conditions this may occur due to heat from a bushfire (Ralph, 1994; Wrigley and Fagg, 1996), by insect action, for example, ants (Blombery and Maloney, 1994), by microbial action in the soil (Wareing and Phillips, 1981), scarification by sand (Blombery and Maloney, 1994), or by repeated cycles of heating and wetting with changes in weather (Wrigley and Fagg, 1996). To mimic these natural conditions, thus removing physical dormancy, seed can be treated in a number of ways.

2.1.1.1.1 Heat treatments

Wet (hot or boiling water) or dry heat can be used. Wet treatments are generally more effective (Ralph, 1994).

2.1.1.1.1.1 Boiling or Hot Water

Boiling water is the most commonly used treatment for hard-seeded species, for example, Acacias (Ralph, 1994). Hot water treatments are also successful and potentially less damaging to the seed (Ralph, 1994). The standard treatment involves pouring boiling water over the seeds and leaving them to soak in the progressively cooling water for 12-24 hr (Hartmann and Kester, 1983; Ralph, 1994; Wrigley and Fagg, 1996; Stewart and Stewart, 1999). The boiling water softens the hard testa, dissolves the waxy coating and allows water to penetrate, thus swelling the seed (Ralph, 1994; Wrigley and Fagg, 1996; Stewart and Stewart, 1999). For hot water treatments, the water should be between 70 – 90°C (Ralph, 1994). Any seed that float after treatment are hollow and therefore, non-viable (Ralph, 1994).

2.1.1.1.1.2 Dry Heat

Seeds can be exposed to periods of dry heat, for example, in an oven, to weaken seedcoats (Ralph, 1994). Microwave ovens can also be used, with treatment times varying from 30-240 sec (Ralph, 1994; Stewart and Stewart, 1999), depending on the moisture content and size of the seed (Stewart and Stewart, 1999). Scorching is another dry heat treatment that can be used to crack the seed coat. Seed should be planted in seedling trays and covered with a thin layer of dry twigs and leaves, shredded paper or straw. The fuel can then be lit and kept burning for approximately 5 min. After the soil has cooled the trays should be well-watered (Stewart and Stewart, 1999).

2.1.1.1.2 Manual Scarification

Hard seedcoats can be manually scarified by a range of methods - the coat can be nicked (Ralph, 1994; Wrigley and Fagg, 1996; Stewart and Stewart, 1999), chipped or pierced (Hartmann and Kester, 1983; Ralph, 1994; Stewart and Stewart, 1999)

with a sharp knife, razor blade or needle (Stewart and Stewart, 1999). A file or sandpaper can also be used to remove a small part of the seedcoat (Hartmann and Kester, 1983; Ralph, 1994; Stewart and Stewart, 1999). In all cases care must be taken to avoid damaging the embryo. Only a small part (no larger than 1 mm square) of the seedcoat should be scarified at the opposite end to the aril (Ralph, 1994).

2.1.1.1.3 Chemical Scarification

Strong acids such as sulphuric or hydrochloric acid have been successfully used to scarify seed coats (Hartmann and Kester, 1983; Blombery and Maloney, 1994; Ralph, 1994). Dry seeds should be placed in containers and covered with the concentrated acid (1 part seed: 2 parts acid). Treatment times will vary according to the thickness of the seed coat (Hartmann and Kester, 1983). Other chemicals including calcium hypochlorite, sodium hydroxide, hydrogen peroxide and ethyl alcohol can also be used (Ralph, 1994). A brief treatment with 5M potassium hydroxide eliminated the physico-chemical barrier that was preventing germination of *Anigozanthos manglesii* (Sukhvibul and Considine, 1994). Fifty percent hydrogen sulphate or 0.7% NaOCl was also effective (*Ibid.*).

2.1.1.1.4 Harvesting Immature Fruits

Harvesting fruit and therefore, seed, before it is fully mature has been successful with some species of *Acacia* and *Hardenbergia*. It improves germination by avoiding the development of hard seed coats (Hartmann and Kester, 1983). This method may also be useful for species without hard seedcoats, but which have other types of dormancy (Ralph, 1994).

Often, combinations of treatments can be even more successful than single treatments. For example, seed may be scarified with sandpaper and then immersed in boiling water (Blombery and Maloney, 1994).

2.1.1.2 Chemical Dormancy and "After-ripening" Periods

Seeds with chemical dormancy are often more difficult to treat than those with physical dormancy (Wrigley and Fagg, 1996). For such seeds, germination will be inhibited until the chemical is removed from the seed. Under natural conditions it is usually a long period of rainfall that leaches the chemical from the seed (Ralph, 1994). The following methods have been used with some success, to mimic natural conditions and remove chemicals from the seed.

2.1.1.2.1 Washing, Soaking or Leaching

For some species the chemicals inhibiting germination can be washed, soaked or leached from the seeds. While the former two treatments are successful for some species, the majority of species with chemical inhibitors require a leaching treatment (Ralph, 1994).

2.1.1.2.1.1 Washing

Seeds are placed in running water and rubbed gently for several minutes (Ralph, 1994).

2.1.1.2.1.2 Soaking

Seeds should be soaked in cold water for no longer than 3 days, and the water should be changed frequently (Ralph, 1994), at least every 12 hr to provide oxygen to the seeds (Hartmann and Kester, 1983). Soaking seed without dormancy mechanisms in water has also resulted in improved germination of some species. It has been suggested that this is due to improved water uptake by the seed. In overseas studies many herbaceous species have benefited from soaking in water for 8 hr (Ralph, 1994). Soaking seed with chemical dormancy in an alkaline solution, with a pH of around 9, has successfully overcome germination inhibitors for species in the Rutaceae family (Ralph, 1994).

2.1.1.2.1.3 Leaching

Seeds should be placed in a permeable bag, for example, muslin or a nylon stocking, and placed under running water for up to 2 weeks (Ralph, 1994; Wrigley and Fagg,

1996; Stewart and Stewart, 1999). The bag can be secured in a suitable container and placed under a running tap (Wrigley and Fagg, 1996); or it can be placed in a toilet cistern, where the seeds will be leached each time the toilet is flushed (Ralph, 1994; Wrigley and Fagg, 1996; Stewart, 1999b).

2.1.1.2.2 Fermentation

Fleshy fruit often contain substances which inhibit the germination of the seed (Blombery and Maloney, 1994). Therefore the flesh should be removed from the seed before sowing. To do this fruit should be sealed in a plastic bag with a small amount of water or wet soil and left to ferment in the sun or some other suitable warm, humid area (Blombery and Maloney, 1994; Ralph, 1994). Once the flesh has fermented, the seed should be washed or leached (Blombery and Maloney, 1994).

2.1.1.2.3 Chemical Treatments

To overcome chemical inhibitors seed can be treated with the chemicals kinetin, thiourea ($CS(NH_2)_2$)or sodium hypochlorite (Ralph, 1994). Kinetin is a cytokinin, a plant growth regulator (PGR) that stimulates cell division in meristem tissue in plants (Dodds and Roberts, 1985). Cytokinins are most effective in breaking dormancy created by chemical inhibitors. Another cytokinin, BAP (at concentrations between 100 and 1000 ppm, or 0.1 - 1.0 g/L) can also be used (Stewart, 1999b). Cytokinins can also be used in combination with gibberellic acid (GA_3) or ethylene-producing compounds (Hartmann and Kester, 1983). Thiourea has a cytokinin-like activity in overcoming inhibition. Seeds can be soaked in a 0.5 - 3% solution for no longer than 24 hr, as it can be inhibitory to growth (Hartmann and Kester, 1983). Chemical treatments can also overcome after-ripening periods. GA_3, potassium nitrate (0.2% solution) and hydrogen peroxide have all been used successfully (Ralph, 1994). GA_3 is a PGR that stimulates growth in seeds and growing tips of plants. Seeds should be soaked in concentrations from 100 to 10,000 ppm (0.1 - 10.0 g/L) (Stewart, 1999b). GA_3 has been successful with exotic species, and initial trials with the native Australian genus *Persoonia* are producing promising results (Wrigley and Fagg, 1996). Up to 40% germination of *Persoonia longifolia* seeds has been achieved

using fresh seed that has been treated with GA_3 after having part of the endocarp chipped away and being sown on the soil surface; control seeds did not germinate (Mullins *et al.*, 2002).

2.1.1.2.4 Storage

For species with after-ripening periods seed will not germinate immediately after collection and must be stored, usually in the dark, for a certain period (Ralph, 1994; Wrigley and Fagg, 1996; Stewart and Stewart, 1999). Optimum storage periods range from 3 - 6 months (Wrigley and Fagg, 1996; Stewart and Stewart, 1999) or even up to 12 months (Ralph, 1994). Storage to allow for after-ripening periods has been successful for many native grasses, herbs, Liliaceae and Epacridaceae species (Ralph, 1994; Wrigley and Fagg, 1996).

2.1.1.2.5 Removal of part of Seedcoat or Fruit

Removing part of the seedcoat or fruit can reduce after-ripening periods, or for species that germinate slowly, germination times can be reduced (Ralph, 1994). For example, some native grasses do not need to be stored when the outer part of the "seed" (which is actually a fruit) is removed (Ralph, 1994). The seed of some species respond well when the entire seedcoat is peeled off (Stewart and Stewart, 1999). This method has been suggested for the native Iridaceae genus, *Patersonia* (Ralph, 1994) and has significantly increased germination percentages of *Hibbertia hypericoides* (Schatral, 1996).

2.1.1.3 Other Seed Treatments

2.1.1.3.1 Stratification

Seeds produced by plants growing in very cold or alpine areas are the most responsive to stratification or "moist chilling" treatments (Bryant, 1985; Stewart and Stewart, 1999). Seed can be planted in a seedling tray, watered and sealed in plastic (Ralph, 1994; Stewart and Stewart, 1999) or it can be mixed in damp sand or moist peat moss, wrapped in plastic and sealed (Wrigley and Fagg, 1996; Stewart and Stewart, 1999). In either case the seed should then be stored in a refrigerator at 2-4°C

(Wrigley and Fagg, 1996) for 3-12 weeks (Stewart and Stewart, 1999). Stratification has been successful for alpine *Eucalyptus* species and could be successful with other alpine genera (Wrigley and Fagg, 1996).

2.1.1.3.2 Bog method

This method is used for species which naturally grow in waterlogged conditions (Ralph, 1994). Seed is sown in a seedling tray which is placed in a tray of water filled to half the depth of the seedling mix (Ralph, 1994).

2.1.1.3.3 Smoke Treatments

Fire has been a part of the Australian environment since at least the Mid to Late Tertiary (Kemp, 1981; Macphail, 1981; Singh *et al.*, 1981) and the many adaptations that plants have evolved to cope with it's effects are numerous within the Australian flora. Therefore, it is perhaps not surprising that seed from species inhabiting fire-prone areas can often be induced to germinate by fire and its associated factors. Germination is thought to be promoted by:

1. Heat from the fire - dry heat causing the testa of hard-seeded species to fracture (Gill, 1975, 1981a,b; Jeffrey *et al.*, 1988) or causing the desiccation of the seed coat (Gill, 1981a,b; Brits *et al.*, 1993), or directly stimulating the embryo to germinate (Van de Venter and Esterhuizen, 1988; Musil and De Witt, 1991);

2. The removal of chemical or microbial inhibitors to germination from the environment (McPherson and Muller, 1969; Kaminsky, 1981; Keeley *et al.*, 1985);

3. Modification of the microenvironment (Baxter *et al.*, 1994) - for example, by mineral enrichment of the soil from ash (Brown, 1993a) and nitrogenous substances (Christensen, 1973; Chambers and Attiwill, 1994; Keeley and Fotheringham, 1997); and by reducing the competition for light (Stewart and Stewart, 1999).

4. Ethylene and/or ammonia present in the smoke (Cairns and De Villiers, 1986; Langkamp, 1987; Van de Venter and Esterhuizen, 1988); and

5. Chemical components in plant-derived smoke and extracts of smoke (De Lange and Boucher, 1990).

Until recently the chemical component/s in smoke that elicited a germination response were unknown. However, recent ground-breaking Australian research (Flematti *et al.*, 2004) has identified the active ingredient. The chemical is a butenolide, made up of a heterocyclic ring of carbons and other atoms (Flematti *et al.*, 2004). It comes from a class of compounds that had never before been found in plants or animals (*Ibid.*).

Following the discoveries that germination of a number of Californian Chaparral species could be enhanced by exposing the seed to charred wood (Keeley *et al.*, 1985), extracts prepared from the charred wood (Keeley and Pizzorno, 1986; Keeley and Keeley, 1987), and that seeds from the rare South African Fynbos species *Audounia capitata* were stimulated to germinate by smoke and smoke extracts (De Lange and Boucher, 1990), the stimulation of seed germination by plant-derived smoke and smoke extracts has been an expanding area of research throughout fire-prone regions of the world.

Research has largely been concentrated in South Africa (Le Maitre and Midgely, 1992; Brown, 1993a, 1993b; Brown *et al.*, 1993, 1994; De Lange and Boucher, 1993; Brown and Botha, 1995; Baxter *et al.*, 1994; Pierce *et al.*, 1995), the Californian Chaparral (Keeley *et al.*, 1985; Keeley and Pizzorno, 1986; Keeley and Fotheringham, 1998) and in Australia, where the majority of work has occurred in Western Australia (Dixon and Roche, 1995; Dixon *et al.*, 1995; Roche *et al.*, 1997a, 1997b; 1998; Lloyd *et al.*, 2000; Tieu *et al.*, 2001) and more recently the eastern states of Victoria (Enright *et al.*, 1997; Enright and Kintrup, 2001), NSW (Read and Bellairs, 1999; Clarke *et al.*, 2000; Morris, 2000; Read *et al.*, 2000), ACT (Willis *et al.*, 2003) and Tasmania (Sward, 1995; Keith, 1997; Marsden-Smedley *et al.*, 1997; Gilmour *et al.*, 2000).

Smoke and smoke extracts have been shown to be key factors in breaking seed dormancy in many native Australian species, that have previously been difficult or impossible to germinate (Ralph, 1994; Dixon and Roche, 1995; Dixon *et al.*, 1995; Keith, 1997; Read and Bellairs, 1999; Smith *et al.*, 1999; Gilmour *et al.*, 2000; Tieu *et al.*, 2001), and as such this method has huge implications for the horticulture and floriculture industries, in conservation of rare and endangered species, and

regeneration of degraded bushland, including old mine sites (Stewart, 1999b; Stewart and Stewart, 1999; Van Staden *et al.*, 2000). The benefits of smoke as a seed pre-treatment include (Dixon and Roche, 1995):

1. Smoke can promote earlier and more uniform germination;
2. Smoke enables germination in species that have previously been found difficult or impossible to germinate by conventional methods;
3. Smoke substantially promotes germination in species with low levels of germination;
4. The promotive effect of smoke is independent of seed size and shape and plant life-form; and
5. Aerosol smoke, smoke dissolved in water or direct smoked solids, or direct smoking of seeds are all effective methods for delivery of smoke for seed germination.

Other suggested benefits include:

1. Smoke acts as a natural pesticide - the application of aerosol smoke to seeds may offer some protection against predation and microbial attack by making seed unpalatable to birds, animals and insects as well as resistant to bacterial attack (Roche *et al.*, 1997b); and
2. The seedlings developing from smoke treated seeds are usually stronger and healthier than those from untreated seeds (Stewart and Stewart, 1999). Smoke may also significantly increase seedling growth rates (Brown, 1993a; Baxter and Van Staden, 1994; Blank and Young, 1998).

However, it should also be noted that:

1. High doses of smoked water can inhibit germination of many species (Dixon and Roche, 1995);
2. Germination over time in response to smoke can change with taxa (Dixon and Roche, 1995);
3. Some species appear to be inhibited by a direct smoke application but are promoted if smoked after sowing in punnets (Dixon and Roche, 1995); and

4. The chemical (butenolide) released by smoke is water-soluble and can be leached from around the seeds if over-watering occurs (De Lange and Boucher, 1993). Therefore, watering should be limited for 24 - 48 hr following smoke treatment (Stewart, 1999b).

Smoke has also been found to sometimes be more successful when used in combination with other treatments, such as heat (Brown *et al.*, 1994a; Keith, 1997; Gilmour *et al.*, 2000; Morris, 2000; Tieu *et al.*, 2001) and also heat + darkness (Keith, 1997; Gilmour *et al.*, 2000). An acid scarification followed by a 24 hr soak in diluted smoke extract increased the germination of the hard-seeded South African legume, *Cyclopia intermedia*, while for *Syncarpha vestita* germination was inhibited by light and promoted by dark. When smoke was used in combination with these factors, the effects of light inhibition were overcome (Stewart, 1999b). Manual scarification followed by soaking in 1% smoke water for 24 hr significantly increased germination percentages of the Liliaceae species *Chamaescilla corymbosa* (Allan *et al.*, 2004). Therefore, for many species a series of environmental cues are required, sometimes in a particular order, while in other species smoke is the main trigger. The natural environment that a species grows in should be examined to determine cues that may interact with smoke treatments (Stewart, 1999b).

2.1.1.3.3.1 Smoke Application Methods

There are a number of ways that smoke can be applied to seeds:

1. Direct smoking of seed (usually for no longer than 1 hr) (Greening Australia, 1996a);
2. Plant seed in a seedling tray and then expose the tray and its contents to smoke (Stewart, 1999b);
3. Soak seed in an aqueous smoke extract (Greening Australia, 1996a; Stewart, 1999b), usually for 6 - 36 hr (Greening Australia, 1996a). For the method used to make smoke extract solutions see Appendix 2.1;
4. Plant seed in a seedling tray and then water with an aqueous smoke extract (Greening Australia, 1996a);

5. Use filter paper, which has been smoked, as a germination base in the laboratory (Greening Australia, 1996a), water with distilled water;

6. For revegetation of areas, the soil can be smoke-fumigated *in situ* using smoke tents (Greening Australia, 1996a; Stewart, 1999b); or

7. Revegetation sites can be irrigated or sprayed with aqueous smoke extracts (Greening Australia, 1996a; Stewart, 1999b).

Seeds can be smoke treated immediately before sowing or they may be treated, dried and stored under appropriate conditions until sown (Dixon and Roche, 1995; Roche *et al.*, 1997b). Aqueous smoke treated seeds of *Syncarpha vestita* (Asteraceae) and *Rhodocoma gigantea* (Restionaceae) can be stored for at least one year without losing the dormancy breaking effect of the smoke pre-soaking treatment (Brown *et al.*, 1994b; Brown and Van Staden, 1999).

A number of commercial "smoke water" solutions are now being produced to ensure that the methods are more readily available for commercial nurserymen and home gardeners. For example, an Australian company with assistance from Kings Park and Botanic Garden, WA has developed Regen 2000®. Three different products are available: Regen 2000 Smokemaster® is suitable for home gardeners, nurseries, Landcare groups etc., while Regen 2000 Direct® and Regen 2000 Seed Starter® are more suited to the restoration of large areas of land (Stewart and Stewart, 1999). Kirstenbosch "Instant Smoke Plus" seed primer, an absorbent paper impregnated with smoke-saturated water, has also been developed in South Africa (D. Orriell Seed Exporters, no date). It is likely that the newly discovered active component of smoke will soon become a commercially available product.

The list of genera and species that are responsive to smoke and smoke extracts is ever-expanding and includes quite a few monocots, some of which are from the native Liliaceae and Iridaceae families. Smoke-responsive Liliaceae and Iridaceae genera (and species, where known) are listed in Table 2.1.

Table 2.1. Native Australian Liliaceae and Iridaceae genera and species (where known) that have shown positive germination responses to smoke and/or smoke extracts. Where a genus only is listed, this does not mean that all species within that genus are smoke-responsive. References used to compile table: Greening Australia (1996a); Stewart and Stewart (1999) and D. Orriell Seed Exporters (no date).

Family	Genus/Species
Liliaceae	*Arthropodium*
	Burchardia umbellata
	Caesia calliantha
	Dianella revoluta
	Sowerbaea laxiflora
	Thysanotus multiflorus
Iridaceae	*Orthrosanthus*
	Patersonia occidentalis

2.1.2 Seed collection and germination of Liliaceae species

2.1.2.1 *Blandfordia*

Blandfordia seeds are contained within a narrow, elongated, triangular (in cross-section) capsule (Stewart and Stewart, 1999). The capsule is dehiscent at maturity, although some of the seed is normally retained within it, until it is dislodged by wind or some other physical action (Wrigley and Fagg, 1996). Therefore, when collecting seed the capsules should be cut carefully (preferably held within a plastic bag) to ensure that no seed is lost (Wrigley and Fagg, 1996). Each chamber within the fruit may hold up to 50 seeds, with an entire stem yielding up to 1000 seeds (Johnson, 1996a). The seed is brown, linear and hairy (Henderson, 1987a; Curtis and Morris, 1994) (Plate 2.1), which may aid in its dispersal (Johnson, 1996a). The literature on seed germination of *Blandfordia* species is consistent, with authors stating that seed is easy to germinate (Blombery, 1972; Collier, 1991; Johnson, 1987, 1990, 1996; Johnson and Burchett, 1991; Stewart and Stewart, 1999; Stewart, 2002) or just that propagation is by seed (Ralph, 1994; Wrigley and Fagg, 1996). Fresh seed is more

viable (Collier, 1991; Stewart and Stewart, 1999; Stewart, 2002) and there is no pre-treatment required (Johnson, 1996a; Stewart and Stewart, 1999) with germination rates being as high as 90% (Johnson, 1990). The seed takes approximately 14 (Wrigley and Fagg, 1996; Stewart and Stewart, 1999) to 21 (Johnson, 1996a; Wrigley and Fagg, 1996; Stewart and Stewart, 1999) days to germinate, and according to Johnson (1996a) should be planted in a mixture of 50% peat and 50% sand, with a pH close to 5.

It is unlikely that there are any dormancy mechanisms present within the *Blandfordia* seed. The seed are soft, so physical dormancy should not be a problem, and there do not appear to be any problems with germination (as noted above).

Plate 2.1. *Blandfordia punicea* seeds.

2.1.2.2 *Dianella*

The *Dianella* fruit is a succulent berry which is usually bright blue (Wrigley and Fagg, 1996). As with most berries, they are mature when they become slightly soft (Wrigley and Fagg, 1996). The seed is black and shiny (Henderson, 1987b) (Plate 2.2). After collection the seed should be spread in drying trays as soon as possible (Wrigley and Fagg, 1996). The literature on seed germination is minimal, with most authors stating only that propagation by seed is possible (Blombery, 1977; Wrigley and Fagg, 1983; Collier, 1990, 1992; Blombery and Maloney, 1994). Elliot and Jones (1984) state that seed-set is frequently low, with some berries not containing

any seed at all. Also, although seeds retain their viability for 12 - 24 months (Elliot and Jones, 1984), they germinate best when sown fresh (Elliot and Jones, 1984; Ralph, 1994). Older seed of *D. revoluta* has indeed failed to germinate (Sophie Juszkiewicz, Kings Park and Botanic Garden, pers. comm., 1995). Germination of *Dianella* seeds has also been noted as being variable (Ralph, 1994). This was found to be the case for *D. tasmanica* with days from sowing to germination ranging from 88 days to 188 days in preliminary seed germination trials (Sward, 1995) and from 47 to 169 days for *D. revoluta* (Sward, 1995). According to Greening Australia (1996b) the number of days to total germination is 83 for *D. tasmanica*, 91 for *D. revoluta* and 129 for *D. longifolia*.

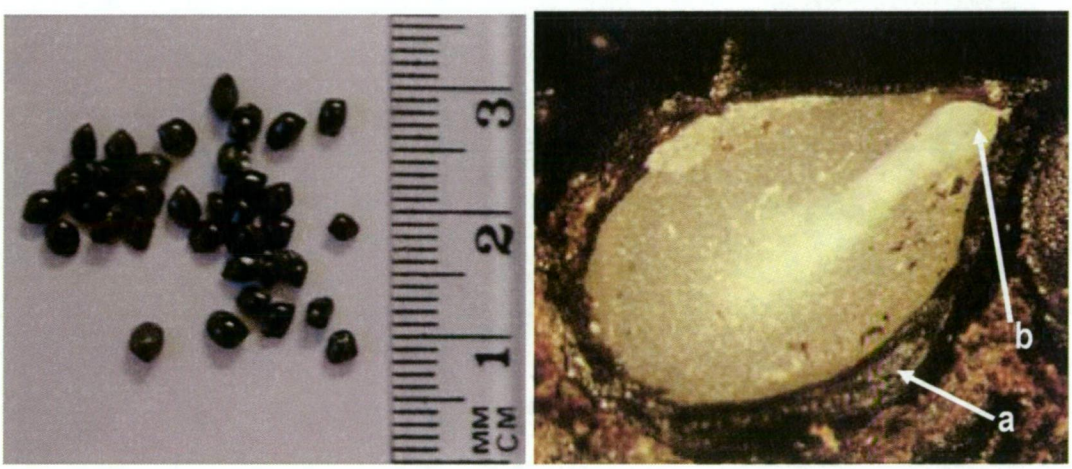

Plate 2.2. *Dianella tasmanica* seeds (left), and cut open seed (right), showing the hard, thick seedcoat (a). Note the white embryo running down the centre of the seed (b).

It is possible that *Dianella* seeds may exhibit physical dormancy, enforced by the hard seedcoat (see Plate 2.2); and perhaps also chemical dormancy due to substances present within the fleshy berry. Seed treatments recommended for improving the germination rate of *Dianella* include, soaking seed in soapy water (using Velvet soap) or giving seed a light scarification with sandpaper (Ralph, 1994). *Dianella* seeds are also responsive to smoke treatments (Kings Park and Botanic Garden, cited in: Stewart and Stewart, 1999), in particular *D. revoluta* (Greening Australia, 1996b).

2.1.2.3 *Milligania*

Milligania seed is held within a 3-valved capsule (George, 1987; Elliot and Jones, 1993; Curtis and Morris, 1994), which is dehiscent at the apex at maturity. The seed itself is black and shiny, with a hard testa (George, 1987). Seed should be collected in the same manner as *Blandfordia* seed. Perhaps due to its endemicity to Tasmania and restricted distribution, the literature about seed germination of this genus is scarce. The only detailed account appears to be by Elliot and Jones (1993), who mention that "there is very little evidence of propagation by seed". They state that seed does not require any treatment prior to sowing, but that stratification for 4 - 6 weeks at approximately 4°C should increase germination rates. The bog treatment may also be beneficial (Elliot and Jones, 1993).

Possible dormancy mechanisms for this genus include physical dormancy that may be imposed by the hard seed coat. Also, as alluded to by Elliot and Jones (1993) who suggest stratification, due to the alpine habitat of this genus seed may require a period of moist chilling prior to germination. The plants usually occur in wet sites (Collier, 1991) so the seed may have adapted to germination under waterlogged conditions, which is why Elliot and Jones (1993) have suggested a bog treatment.

2.1.3 Seed collection and germination of Iridaceae species

2.1.3.1 *Diplarrena*

The *Diplarrena* fruit is a 3-chambered capsule (Rodway, 1922; Elliot and Jones, 1984; Cameron, 1992; Curtis and Morris, 1994) (Plate 2.3). The seeds are flat (Rodway, 1922; Cooke, 1986; Curtis and Morris, 1994), disk-like and brown (Plate 2.3). Although only about 4 capsules on any inflorescence will reach maturity (Curtis and Morris, 1994), the seed per capsule is numerous (Rodway, 1922; Cooke, 1986; Curtis and Morris, 1994). Seed should be collected in the same way as *Blandfordia* and *Milligania*. The references to seed germination for this genus state that propagation is by seed (Elliot and Jones, 1984; Collier, 1990; Ralph, 1994; Wrigley and Fagg, 1996), but no further details are given.

Plate 2.3. *Diplarrena moraea* seed capsules (left) and seeds (right). The embryo (a) runs down the centre of the seed. Scale bar = 4 mm.

It is unlikely that there would be any dormancy mechanisms present in this genus. The seed does not have a hard coat, indeed it is quite flat and the embryo is visible through the seed coat, which suggests that it may be prone to desiccation and thus not be viable for very long.

2.1.3.2 *Isophysis*

The *Isophysis* fruit is an ovoid, triangular (in cross-section) capsule (Cooke, 1986; Curtis and Morris, 1994), which dehisces at maturity. The seeds are small, angular and light brown (Cooke, 1986; Curtis and Morris, 1994) (Plate 2.4), and they are numerous within the capsule (Cooke, 1986; Cameron, 1992). Seed should be collected in the same manner as *Blandfordia* and *Milligania*. As for *Milligania* there is very little literature about seed germination of *Isophysis*, probably due also to its endemicity to Tasmania and restricted distribution. The genus can be propagated by seed (Ralph, 1994; Wrigley and Fagg, 1996) but no further information is given. A general time frame for germination for Iridaceae species is 14 - 21 days (Wrigley and Fagg, 1996), but no specific time for *Isophysis* seeds could be found.

It is not likely that any dormancy mechanisms would be present within *Isophysis* seeds, however, due to their alpine habitat they may benefit from a period of stratification.

Plate 2.4. *Isophysis tasmanica* seeds.

2.2 Materials and Methods

2.2.1 General Materials and Methods

The following information relates to all seed germination experiments. If any details vary this will be noted in the relevant section.

- Seeds were checked at least once every 2 days until germination had begun. They were then checked weekly and the number of germinants in each treatment were counted and recorded to allow germination rates and percentages to be determined.
- Seeds were considered to be germinated when any part of the radicle was exposed.

- For all petri dish experiments (*ex vitro*), seeds were watered with distilled water unless otherwise stated. Throughout the duration of the experiment, seeds were watered when required, with the filter paper kept moist, but not saturated.
- When seeds were placed in dark conditions, petri dishes or culture tubes were wrapped in aluminium foil or placed in a light-proof, sealed cardboard box.
- The smoke extract solutions were obtained by a similar method to De Lange and Boucher (1990). See Appendix 2.1 for details. Smoke extract solutions were used at the full concentration achieved from this method (100% smoke) or were diluted to the appropriate concentration by adding distilled water.
- For all *in vitro* experiments, culture tubes containing individual seeds were placed in an incubation room with a controlled temperature of 25 ± 2°C, a 16 hr photoperiod and a photonfluence of 15 - 18 $\mu Em^{-2}s^{-1}$.
- The *ex vitro* experiments that were placed in the incubation room to germinate were also subject to the same conditions.
- For all *in vitro* experiments, the surface sterilisation procedure involved placing the seeds in 30 mL polycarbonate tubes (no more than 5 seeds per tube), and adding a sodium hypochlorite (NaOCl) solution, at the appropriate concentration, until the seeds were completely covered. They were agitated in the tubes to ensure that all seed surfaces came into contact with the solution. Seeds remained in NaOCl for the appropriate time. After sterilisation, NaOCl was decanted from the tubes under sterile conditions in a laminar flow cabinet, and the seeds were rinsed twice with sterile distilled water.
- Data analysed by ANOVA and Tukey's HSD tests were performed using the SPSS for Windows statistical package (Version 10) (SPSS Incorporated, 2000).

2.2.2 Liliaceae Seed Germination Experiments

2.2.2.1 *Blandfordia punicea*

2.2.2.1.1 *B. punicea* smoke solution experiment

Seed capsules were collected at different levels of maturity from a coastal population of *Blandfordia punicea* at South Cape, Tasmania. Depending on maturity level the seeds were labelled as follows:

South Cape 1 - the most mature capsule, which was still green but had begun to fade to yellow and appeared to be starting to dry out. The seeds were brown and appeared to be mature, but felt soft (collected in late January);

South Cape 2 - Capsules were still green, and seeds were brown, as above (collected in mid-December); and

South Cape 3 - Capsules were purple-green and appeared to be still developing. The seeds were green - light brown and were definitely immature (collected in mid-December).

Seeds were placed on 2 layers of Whatman No. 1 filter paper in plastic petri dishes and watered with the solutions shown in Table 2.2. Due to limited seed numbers, especially in the two immature seed lots, the number of seeds per treatment was quite low (Table 2.2). Petri dishes were placed on a window ledge in the laboratory to germinate.

Pearson's Chi-squared statistic and pairwise comparisons were used to compare percentage germination data for all seed lots and treatments.

Table 2.2. Treatments that *B. punicea* seeds were watered with and the number of seeds used from each seed lot for those treatments. Rep./s = Replicate/s.

Treatment	Number of seeds from each seed lot		
	SC1	SC2	SC3
1. Control - Distilled water (Light)	20 (2 reps. of 10)	1 rep. of 10	1 rep. of 10
2. Control - Distilled water (Dark)	20 (2 reps. of 10)	1 rep. of 10	1 rep. of 10
3. 10% smoke solution (Light)	20 (2 reps. of 10)	1 rep. of 10	1 rep. of 10
4. 10% smoke solution (Dark)	20 (2 reps. of 10)	1 rep. of 10	1 rep. of 10
5. 50% smoke solution (Light)	20 (2 reps. of 10)	1 rep. of 10	1 rep. of 10
6. 50% smoke solution (Dark)	20 (2 reps. of 10)	1 rep. of 10	1 rep. of 10
7. 100% smoke solution (Light)	20 (2 reps. of 10)	1 rep. of 10	1 rep. of 10
4. 100% smoke solution (Dark)	20 (2 reps. of 10)	1 rep. of 10	1 rep. of 10

2.2.2.1.2 *B. punicea in vitro* light/ dark experiment

This experiment also utilised the three seed lots collected from South Cape, as described above. The experiment was performed under *in vitro* conditions to determine any differences in germination behaviour on different media types in combination with light and dark conditions. The media types used were: a basal MS medium (Murashige and Skoog, 1962) (see Appendix 2.2) and an embryo culture (EC) medium (see Appendix 2.3), which has been used successfully with *Nerine* seed (Dr Natalie Brown, pers. comm., 1997). Seeds were surface sterilised in NaOCl for 10 min. The mature seeds (SC1) were sterilised in 2% NaOCl, while the immature seeds (SC2 and SC3) were placed in 1% NaOCl. Following sterilisation and rinsing, the seeds were placed randomly in tubes of the appropriate media, with one seed per tube. All tubes were placed in an incubation room to germinate. The number of seeds used in each treatment is shown in Table 2.3.

Data were analysed using Pearson's Chi-Squared Statistic and pairwise comparisons.

Table 2.3. Number of *B. punicea* seeds used in each *in vitro* treatment.

Seed lot	Media type	Number of seeds in dark conditions	Number of seeds in light conditions
SC1	MS	20	20
SC1	EC	20	20
SC2	MS	20	20
SC2	EC	20	20
SC3	MS	20	20
SC3	EC	20	20

2.2.2.1.3 *B. punicea in vitro* media type and kinetin experiment

The seeds used in this experiment were all collected from the same plant, with a yellow/orange flower colour, from the same South Cape population described in the two previous experiments. Collection time was late January. Seed was placed on 3 different basal media to determine any differences in germination patterns. MS medium was also supplemented with 5 different concentrations of the cytokinin kinetin, to determine whether this PGR could increase the natural germination rate of *B. punicea* seeds. Before placement on each media type, seed was sterilised in 2% NaOCl for 10 min and placed randomly in tubes of the appropriate media. The number of seeds placed in each treatment is listed in Table 2.4.

The percentage germination data and the percentage of seedlings that died after germinating was analysed using Pearson's Chi-Squared statistic and pairwise comparisons.

Table 2.4. Number of *B. punicea* seeds placed on each media treatment *in vitro*. EC = embryo culture medium, VFT = Venus Fly Trap medium.

Treatment	Number of seeds
1. MS	60
2. EC	60
3. VFT	60
4. MS + 0.5μM kinetin	20
5. MS + 2μM kinetin	20
6. MS + 8μM kinetin	20
7. MS + 32μM kinetin	20
8. MS + 128μM kinetin	20

2.2.2.2 *Dianella tasmanica*

2.2.2.2.1 *D. tasmanica* oven experiment

D. tasmanica seeds were heated in an oven at different temperatures (See Table 2.5) for 10 min, placed on two layers of Whatman No.1 filter paper in plastic petri dishes and watered with distilled water. There were 5 replicates of 10 seeds in each treatment. Petri dishes containing seeds were kept at ambient temperature in the light on window ledges in a laboratory.

Final germination results were analysed by ANOVA and Tukey's HSD Tests.

Table 2.5. Oven treatments used for *D. tasmanica* seeds.

Treatment	Description
1. Control	50 seeds were untreated
2. Oven 50°C	50 seeds were placed in an oven at 50°C for 10 min
3. Oven 80°C	50 seeds were placed in an oven at 80°C for 10 min
4. Oven 100°C	50 seeds were placed in an oven at 100°C for 10 min

2.2.2.2.2 *D. tasmanica* fermentation experiment

Freshly collected *D. tasmanica* fruit were separated into two groups. Half were placed into a ziplock plastic bag with about 5 mL of water. The bag was sealed and placed into the incubation room at a constant temperature of 25°C. The other half was placed onto paper towel to dry at room temperature. After approximately two weeks, the seeds were removed from the fruit, rinsed with tap water in a fine sieve and placed onto newspaper to dry. Seeds were put onto two layers of Whatman No.1 filter paper in plastic petri dishes and watered with distilled water. There were 9 replicates of 20 seeds in both the control and fermentation treatments.

Petri dishes containing seeds were kept at ambient temperature in the light on window ledges in a laboratory.

The results were analysed by ANOVA.

2.2.2.2.3 *D. tasmanica* smoke experiment

D. tasmanica seeds were either watered with a smoke extract solution (at various concentrations) (see Appendix 2.1 for details on the preparation of this solution) or direct smoked to compare these methods of smoke application on the germination behaviour of this species. The treatments applied and the number of seeds in each treatment are outlined in Table 2.6.

Table 2.6. Smoke treatments applied to *D. tasmanica* seeds.

Treatment	Description	Number of seeds	Number of replicates
1. Control	Seeds watered with distilled water	60	6 replicates of 10 seeds
2. Direct smoke	Seeds placed in a plastic zip-lock bag and smoke (generated by burning slightly damp leaf litter and dead twigs of native vegetation in an apiarist's smoker) was puffed into the bag for 5 min. The bag was sealed and left for 2.5-3 hr. Seeds were removed, placed in the petri dishes and watered with distilled water.	60	6 replicates of 10 seeds
3. 1% smoke extract	Seeds watered with a smoke solution diluted to 1% of the original concentration	60	6 replicates of 10 seeds
4. 10% smoke extract	Seeds watered with a smoke solution diluted to 10% of the original concentration	60	6 replicates of 10 seeds
5. 50% smoke extract	Seeds watered with a smoke solution diluted to 50% of the original concentration	60	6 replicates of 10 seeds
6. 100% smoke extract	Seeds watered with an undiluted smoke solution	60	6 replicates of 10 seeds

The seeds were placed in plastic petri dishes on two layers of Whatman No.1 filter paper (prior to treatment for smoke extract treated seeds, and following treatment for direct smoked seeds). The petri dishes were placed randomly together on a window ledge in a laboratory. Throughout the duration of the experiment seeds were watered with distilled water (control and direct smoke treatments) or the appropriate

concentration of smoked water when required. The filter paper was kept moist, but not saturated.

Final germination results were analysed by ANOVA and Tukey's HSD tests.

2.2.2.2.4 *D. tasmanica* partial removal of testa experiment

Sixty *D. tasmanica* seeds were surface sterilised for 20 min in 2% NaOCl and rinsed twice in SDW. Half of the seeds had their testa nicked using a sterile scalpel and part of the seed coat was removed. The other half remained untreated (control). Seeds were placed individually in tubes of MS media, which were placed in an incubation room. All seeds were checked daily for germination.

The percentage germination data was analysed using Pearson's Chi-Squared statistic.

2.2.2.3 *Milligania densiflora*

2.2.2.3.1 Initial *M. densiflora* seed germination experiment

A simple experiment was performed to determine the natural germination rate of *M. densiflora* seeds. Two different seed lots were used, one that was obtained from the Melbourne Botanic Gardens (MBG) in 1996 (collection date not available) and was at least two years old, and one from Mt Read, Tasmania (collected approximately 4 months before the experiment began). Seeds, without any pre-treatment, were placed in petri dishes on two layers of Whatman No. 1 filter paper and watered with distilled water. Approximately half of the seeds were placed in an incubation room, while the rest were placed on a window ledge in the laboratory under ambient conditions. The number of seeds used for each treatment is shown in Table 2.7. Numbers used from MBG were less, due to limited seed available.

The final germination results were analysed using Pearson's Chi-Squared statistic and pairwise comparisons.

Table 2.7. The number of *M. densiflora* seeds from each seed lot used for each treatment.

Seed Location	Number of seeds in laboratory	Number of seeds in incubation room
1. Melbourne Botanic Gardens	10	10
2. Mt Read, Tasmania	44 (2 petri dishes of 22)	44 (2 petri dishes of 22)

2.2.2.3.2 Seed germination of *M. densiflora in vitro*

A second experiment using the same seed as above was run concurrently, with seeds being placed *in vitro* to determine their responsiveness to these conditions. Using sterile techniques, seeds were surface sterilised in 2% NaOCl for 15 min, before being placed in tubes of MS media. The tubes were placed in an incubation room to germinate. Twenty four seeds from MBG were used and 63 seeds from Mt Read. Due to problems with contamination results were not analysed statistically.

2.2.2.3.3 Seed germination of *M. densiflora in vitro* when disinfested for different times and placed on two media types

After the very high contamination rates experienced in the previous experiment, a second experiment was devised to try to overcome this problem. Seeds from Mt Read, Tasmania were disinfested in 2% NaOCl for either 20 or 35 min (as the 15 min used in the previous experiment was not long enough to thoroughly remove all contaminants on the seed surfaces). Also, because a high proportion of the seedlings in the previous experiment died shortly after germinating, it was suspected that the nutrient concentrations within the MS medium may have been too strong. In its natural habitat *M. densiflora* grows in nutrient poor soils, often in cracks between rocks where there is very little soil present (Curtis and Morris, 1994; Kirkpatrick, 1997). Therefore, two different strengths of media were used, a normal strength MS, and a half-strength (1/2) MS. The number of seeds used in each treatment is shown in Table 2.8.

Following surface sterilisation and rinsing, seeds were placed randomly onto the appropriate media type, under sterile conditions. Culture tubes containing individual seeds were placed in an incubation room to germinate.

The final germination results, the percentage of seeds that became contaminated and the percentage of seedlings that died after germination were analysed using Pearson's Chi-Squared statistic, and pairwise comparisons.

Table 2.8. The number of *M. densiflora* seeds in each media and disinfestation treatment *in vitro*.

Medium Type	20 Min Disinfestation	35 Min Disinfestation
1. MS	60	60
2. 1/2 MS	60	60

2.2.3 Iridaceae seed germination experiments

2.2.3.1 *Diplarrena moraea*

2.2.3.1.1 *D. moraea* initial *in vitro* seed germination experiment

An initial experiment was performed to determine seed germination behaviour of *D. moraea in vitro*. The date of seed collection was unknown (it was not collected by the author), and it had been stored for some time. As *Diplarrena* seed is quite thin and possibly easily damaged, two concentrations of NaOCl were used to disinfest the seeds, a weak 1% solution and a stronger 2% solution.

Twenty four seeds were surface sterilised for 15 min, with 12 disinfested in 1% NaOCl and the other 12 in 2% NaOCl. The seeds were placed on MS medium, with 1 seed per 30 mL tube. The tubes were placed in an incubation room to germinate.

2.2.3.1.2 *D. moraea* second *in vitro* seed germination experiment

Due to the poor results from the first experiment with *D. moraea* (above), a second experiment was performed. This time very fresh seed collected from Mt Nelson, Tasmania was used. Thirty three seeds were all disinfested with 2% NaOCl for 20 min and placed in 30 mL tubes of MS medium, with 1 seed per tube. They were put in an incubation room to germinate.

2.2.3.2 *Diplarrena latifolia*

2.2.3.2.1 *D.latifolia in vitro* media type and kinetin experiment

This experiment was designed to test the response of *D. latifolia* seeds to two basal media types, MS and EC, in light and dark conditions. In addition, seeds were grown on MS supplemented with five different concentrations of kinetin to determine their germination response to this PGR.

Due to the successful disinfestation in the second *D. moraea* experiment above, *D. latifolia* seeds were also disinfested with 2% NaOCl for 20 min. Following sterilisation and rinsing, individual seeds were placed in 30 mL tubes of 9 different media types (Table 2.9). Tubes containing seeds were placed in an incubation room to germinate.

The final germination results and the percentage of seedlings that died after germination in each treatment were analysed using Pearson's Chi-Squared statistic and pairwise comparisons.

Table 2.9. Media treatments and conditions that *D. latifolia* seeds were grown on *in vitro*.

Treatment	Number of seeds
1. MS (light)	35
2. MS (dark)	35
3. EC (light)	25
4. EC (dark)	25
5. MS + 0.5μM kinetin (light)	20
6. MS + 2μM kinetin (light)	20
7. MS + 8μM kinetin (light)	20
8. MS + 32μM kinetin (light)	20
9. MS + 128μM kinetin (light)	20

2.2.3.3 *Isophysis tasmanica*

2.2.3.3.1 *I. tasmanica* light/dark experiment *in vitro*

This small experiment was done to determine the germination behaviour of *I. tasmanica* seeds *in vitro* under light and dark conditions. Forty seeds were disinfested with 2% NaOCl for 15 min and placed in 30 mL tubes of MS medium, with 1 seed per tube. All of the seeds were placed in an incubation room to germinate. Half of the seeds were grown in the light, while the other half were grown in the dark.

The final germination results were analysed using Pearson's Chi-Squared statistic.

2.2.3.3.2 *I. tasmanica* smoke solution experiment

I. tasmanica seeds were placed in plastic petri dishes on two layers of Whatman No.1 filter paper and watered with different concentrations of a smoke extract solution (for details on the preparation of this solution see Appendix 2.1). Half of the seeds from each treatment were grown in the light, while the other half were grown in the dark. The petri dishes were placed randomly together on a window ledge in a laboratory. The treatments applied to seeds and the number of seeds in each treatment are outlined in Table 2.10.

Throughout the duration of the experiment, seeds were watered with distilled water (control) or the correct concentration of smoked water when required. The filter paper was kept moist, but not saturated.

Final germination results were analysed by ANOVA.

Table 2.10. Smoke solution treatments applied to *I. tasmanica* seeds.

Treatment	Description	Number of seeds	Number of replicates
1. Control (light)	Seeds watered with distilled water and kept in the light	20	2 replicates of 10 seeds
2. Control (dark)	Seeds watered with distilled water and kept in the dark	20	2 replicates of 10 seeds
3. 10% smoke (light)	Seeds watered with a smoke solution diluted to 10% of the original concentration and kept in the light	20	2 replicates of 10 seeds
4. 10% smoke (dark)	Seeds watered with a smoke solution diluted to 10% of the original concentration and kept in the dark	20	2 replicates of 10 seeds
5. 50% smoke (light)	Seeds watered with a smoke solution diluted to 50% of the original concentration and kept in the light	20	2 replicates of 10 seeds
6. 50% smoke (dark)	Seeds watered with a smoke solution diluted to 50% of the original concentration and kept in the dark	20	2 replicates of 10 seeds
7. 100% smoke (light)	Seeds watered with the undiluted smoke solution and kept in the light	20	2 replicates of 10 seeds
8. 100% smoke (dark)	Seeds watered with the undiluted smoke solution and kept in the dark	20	2 replicates of 10 seeds

2.3 Results

2.3.1 Liliaceae Seed Germination Experiments

2.3.1.1 *Blandfordia punicea*

2.3.1.1.1 *B. punicea* smoke solution experiment

Pearson's Chi-squared statistic showed that there were differences between treatments in respect of percentage germination ($\chi^2 = 246.7$; $p = 0.00$).

None of the seeds from the SC2 seed lot germinated in any of the treatments, and as such the germination percentages of this seed lot were significantly different to those of the SC1 seed treatments ($p = 0.00$) and the majority of the SC3 seed treatments ($p = 0.00$ or $p = 0.03$), with the exception of the 100% smoke light and 100% smoke dark treatments (Table 2.11).

Seeds from the SC1 seed lot had a higher germination percentage in all treatments than the SC3 seed lot. Pairwise comparisons showed significant differences between nearly all SC1 and SC3 treatments ($p < 0.05$) with some exceptions (Table 2.11). The maximum germination percentage for SC1 was 100%, which occurred in 4 treatments (control light, 10% smoke light, 10% smoke dark and 50% smoke dark), and the other 4 treatments reached 95% (Fig. 2.1). For seeds from the SC3 seed lot, 70% was the highest germination reached (in 3 treatments: control dark, 10% smoke dark and 50% smoke light) and the lowest was 0%, in the 100% smoke light treatment (Fig. 2.2).

There were no differences in percentage germination between treatments for SC1 seeds ($p > 0.05$), however for SC3, germination was significantly inhibited by the 100% smoke solution in both light and dark treatments, when compared to the other treatments ($p < 0.05$), with the exception of the comparison between control light and 100% smoke dark ($p > 0.05$). However, the smoke solution appeared to have a less inhibitory effect when seeds were kept under dark conditions (n.s.). The control seeds also germinated better in the dark (n.s.). Apart from the two 100% smoke

treatments, germination percentages were higher than the control light seeds in all other treatments for the SC3 seed lot. However, differences were not significant.

Table 2.11. p-values for pairwise comparisons of percentage germination for all *B. punicea* smoke solution treatments, using Chi-Squared Tests. Significant values ($p < 0.05$) are highlighted.

	p-values																					
T	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
1	.31	1.0	1.0	.31	1.0	.31	.31	.00	.00	.00	.00	.00	.00	.00	.00	.00	.01	.00	.01	.01	.00	.00	.00
2		.31	.31	1.0	.31	1.0	1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.05	.02	.05	.05	.02	.00	.00
3			1.0	.31	1.0	.31	.31	.00	.00	.00	.00	.00	.00	.00	.00	.00	.01	.00	.01	.01	.00	.00	.00
4				.31	1.0	.31	.31	.00	.00	.00	.00	.00	.00	.00	.00	.00	.01	.00	.01	.01	.00	.00	.00
5					.31	1.0	1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.05	.02	.05	.05	.02	.00	.00
6						.31	.31	.00	.00	.00	.00	.00	.00	.00	.00	.00	.01	.00	.01	.01	.00	.00	.00
7							1.0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.05	.02	.05	.05	.02	.00	.00
8								.00	.00	.00	.00	.00	.00	.00	.00	.00	.05	.02	.05	.05	.02	.00	.00
9									1.0	1.0	1.0	1.0	1.0	1.0	1.0	.03	.00	.00	.00	.00	.00	1.0	.31
10										1.0	1.0	1.0	1.0	1.0	1.0	.03	.00	.00	.00	.00	.00	1.0	.31
11											1.0	1.0	1.0	1.0	1.0	.03	.00	.00	.00	.00	.00	1.0	.31
12												1.0	1.0	1.0	1.0	.03	.00	.00	.00	.00	.00	1.0	.31
13													1.0	1.0	1.0	.03	.00	.00	.00	.00	.00	1.0	.31
14														1.0	1.0	.03	.00	.00	.00	.00	.00	1.0	.31
15															1.0	.03	.00	.00	.00	.00	.00	1.0	.31
16																.03	.00	.00	.00	.00	.00	1.0	.31
17																	.18	.37	.18	.18	.37	.03	.12
18																		.64	1.0	1.0	.64	.00	.01
19																			.64	.64	1.0	.00	.02
20																				1.0	.64	.00	.01
21																					.64	.00	.01
22																						.00	.02
23																							.31

T1 = SC1 Control Light
T2 = SC1 Control Dark
T3 = SC1 10% Smoke Light
T4 = SC1 10% Smoke Dark
T5 = SC1 50% Smoke Light
T6 = SC1 50% Smoke Dark
T7 = SC1 100% Smoke Light
T8 = SC1 100% Smoke Dark
T9 = SC2 Control Light
T10 = SC2 Control Dark
T11 = SC2 10% Smoke Light
T12 = SC2 10% Smoke Dark
T13 = SC2 50% Smoke Light
T14 = SC2 50% Smoke Dark
T15 = SC2 100% Smoke Light
T16 = SC2 100% Smoke Dark
T17 = SC3 Control Light
T18 = SC3 Control Dark
T19 = SC3 10% Smoke Light
T20 = SC3 10% Smoke Dark
T21 = SC3 50% Smoke Light
T22 = SC3 50% Smoke Dark
T23 = SC3 100% Smoke Light
T24 = SC3 100% Smoke Dark

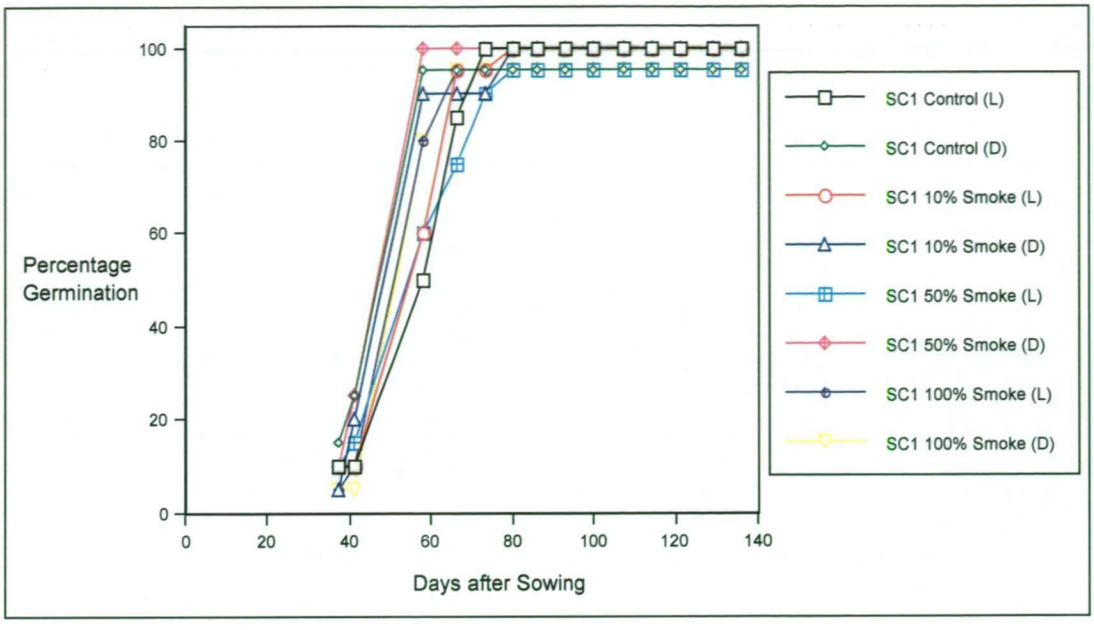

Figure 2.1. Percentage germination of *B.punicea* SC1 seeds treated with different concentrations of smoke extract solution and light/dark regimes. L = light; D = dark.

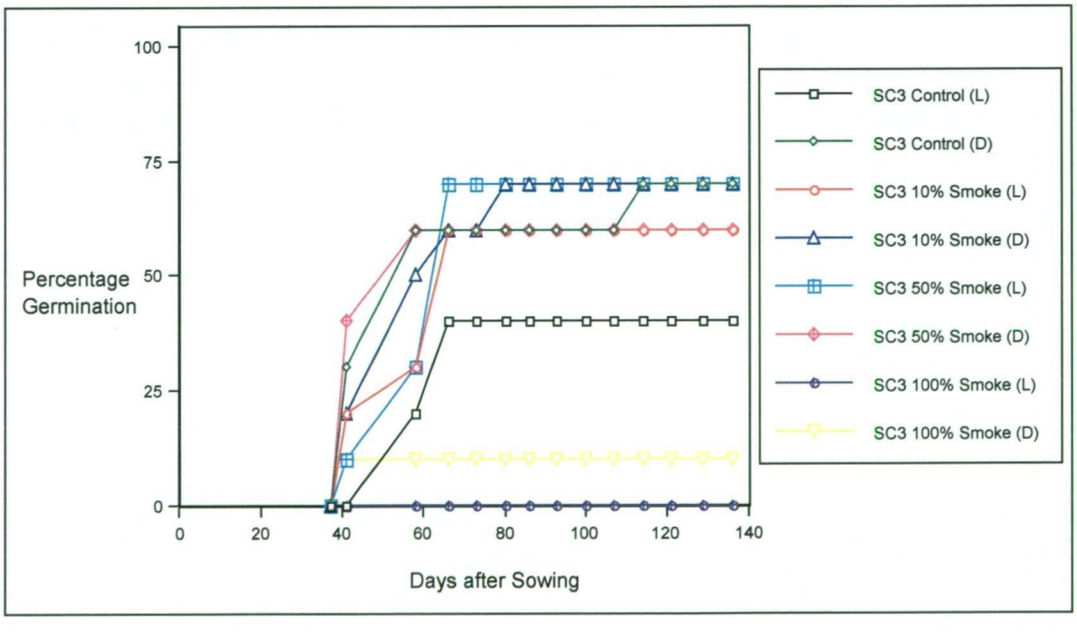

Figure 2.2. Percentage germination of *B.punicea* SC3 seeds treated with different concentrations of smoke extract solution and light/dark regimes. L = light; D = dark.

In terms of time to germination, the SC1 seeds began to germinate slightly earlier than the SC3 seeds. Thirty seven days after sowing (DAS), between 5 and 15% of SC1 seeds had germinated, but SC3 seeds did not start to germinate until 41 DAS.

However, the percentage germination at this time was generally higher than the comparable treatments for SC1 seeds (Figs 2.1, 2.2).

It took between 41 and 114 days for maximum germination to be reached, with seeds from all treatments, except for SC3 control (dark), reaching their capacity by 80 DAS. SC1 seeds took 58 to 80 days to reach maximum germination, while SC3 seeds took 41 to 114 days. The seeds that reached their germination capacity fastest were all in dark treatments, with 100% smoke dark SC3 seeds only taking 41 days (however, only 10% of seeds germinated). Perhaps the most successful treatment was SC1 50% smoke (dark), where 100% of seeds germinated in just 58 days (Fig. 2.1). The SC1 control (dark) seeds also reached their germination capacity of 95% in the same time (Fig. 2.1), as did SC3 50% smoke (dark) treated seeds (with a maximum of 60% germination) (Fig. 2.2).

2.3.1.1.2 *B. punicea in vitro* Light/ Dark Experiment

Pearson's Chi-Squared test showed differences between treatments in respect of percentage germination ($\chi^2 = 159.2$; $p = 0.00$).

As was the case in the previous experiment, none of the seeds from the SC2 seed lot germinated in any of the treatments, and as such the percentage germination of all SC2 seed treatments was significantly different to those from the SC1 and SC3 seed lots ($p = 0.00$ for all pairwise comparisons between these treatments, see Table 2.12).

Seed germination percentages were greater than 50% for all SC1 and SC3 treatments, with the highest percentage (100%) reached by the SC1 seeds on MS in both light and dark conditions, and the SC3 seeds in the EC light treatment (Fig. 2.3). There were no significant differences between the percentage values of seeds under light and dark conditions when seeds were grown on MS media (SC1: $p = 1.0$; SC3: $p = 0.71$), but when grown on EC media, germination was better in the light than the dark, for both seed lots (SC1: n.s; SC3: $p = 0.00$).

Seeds began to germinate 36 days after placement on the media in all treatments except for the EC dark treatment, where both SC1 and SC3 seeds did not begin to germinate until slightly later (41 days after sowing) (Fig. 2.3). The germination rate

was quite rapid, with 4 treatments reaching maximum germination only 41 DAS, the best of these being MS dark for SC1 and EC light for SC3, where seeds reached 100% germination at this time (Fig. 2.3). The other treatments that reached maximum germination at this time were EC dark for both seed lots, where the maximum germination was 50%, which was the lowest for all treatments. The range to maximum germination was smaller for SC1 seed treatments (41-86 days), compared to 41-107 days for the SC3 seeds.

MS media was a more successful treatment (in terms of percentage germination) for the more mature SC1 seeds ($p < 0.05$, Table 2.12), while EC media was more successful for SC3 seeds, but only under light conditions ($p = 0.04$, Table 2.12). In terms of time to maximum germination, for SC1 seeds dark conditions were better on both media types, while for SC3 seeds, EC media produced a faster germination rate (in both light and dark) than MS media (Fig. 2.3).

Table 2.12. p-values for pairwise comparisons of percentage germination for all *B. punicea* light and dark treatments *in vitro*, using Chi-Squared tests. Significant values ($p < 0.05$) are highlighted.

	p-values										
Treatment	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	1.00	0.04	0.00	0.00	0.00	0.00	0.00	0.04	0.02	1.0	0.00
2		0.04	0.00	0.00	0.00	0.00	0.00	0.04	0.02	1.0	0.00
3			0.05	0.00	0.00	0.00	0.00	1.00	0.71	0.04	0.05
4				0.00	0.00	0.00	0.00	0.05	0.10	0.00	1.00
5					1.00	1.00	1.00	0.00	0.00	0.00	0.00
6						1.00	1.00	0.00	0.00	0.00	0.00
7							1.00	0.00	0.00	0.00	0.00
8								0.00	0.00	0.00	0.00
9									0.71	0.04	0.05
10										0.02	0.10
11											0.00

T1 = SC1 MS light
T2 = SC1 MS dark
T3 = SC1 EC light
T4 = SC1 EC dark
T5 = SC2 MS light
T6 = SC2 MS dark
T7 = SC2 EC light
T8 = SC2 EC dark
T9 = SC3 MS light
T10 = SC3 MS dark
T11 = SC3 EC light
T12 = SC3 EC dark

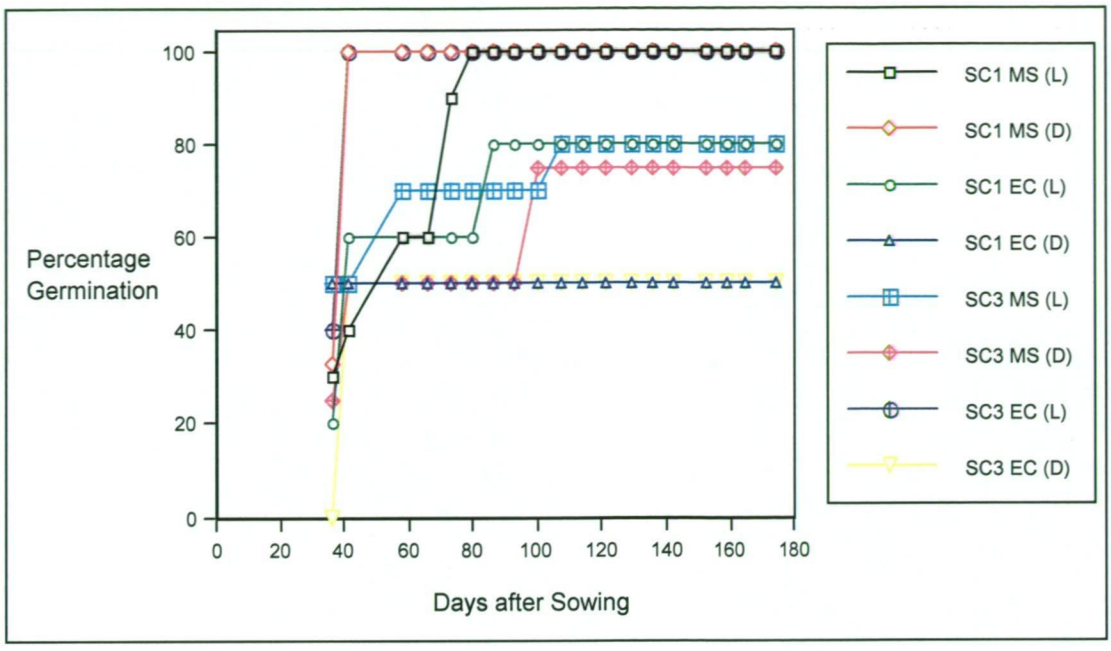

Figure 2.3. Percentage germination of *B.punicea* seeds *in vitro* on MS and EC media, under light and dark conditions, at the days after sowing shown. L = light; D = dark. SC2 treatments have not been included as seeds did not germinate in any of these treatments.

2.3.1.1.3 *B. punicea in vitro* media type and kinetin experiment

Pearson's Chi-Squared test showed that there were differences between treatments in respect to percentage germination ($\chi^2 = 23.39$; $p = 0.0002$).

Germination percentages were very high when seeds were grown on the majority of media types tested, with 4 treatments reaching 100% germination (Fig. 2.4). Three of these media types were supplemented with kinetin (at concentrations of 0.5μM, 32μM and 128μM) and the other was the VFT medium (a half strength MS medium containing another cytokinin, 2iP). The lowest germination of 67% also occurred on a medium with kinetin (8μM), all other treatments achieved greater than 88% germination (Fig. 2.4), and were significantly different to this treatment ($p < 0.05$) with the exception of EC and MS + 2μM kinetin ($p > 0.05$, Table 2.13).

Seeds grown on the VFT medium produced significantly higher germination percentages than those grown on EC medium ($p = 0.01$), MS + 2μM kinetin ($p = 0.03$) and MS + 8μM kinetin ($p = 0.00$).

Although the addition of kinetin to the media generally increased germination percentages compared to the control, this difference was not significant (Table 2.13). The percentage germination of control seeds was only significantly different, and higher, than the MS + 8μM kinetin treatment ($p = 0.01$), which had the lowest percentage germination of all treatments.

In regard to the kinetin treatments, the 3 treatments that reached 100% germination (MS + 0.5μM kinetin, MS + 32μM kinetin and MS + 128μM kinetin) were significantly higher than the MS + 8μM kinetin treatment ($p = 0.01$ in all comparisons), but not than the MS + 2μM kinetin treatment.

Seeds began to germinate 52 DAS on all media types except for: EC, MS + 2μM kinetin and MS + 8μM kinetin. EC and MS + 2μM kinetin treatments took only 1 week longer to begin germinating (59 DAS), while the seeds on the MS + 8μM kinetin treatment did not start to germinate until 73 DAS (Fig. 2.4).

There was quite a wide range in times taken to reach maximum germination, between 88 days after sowing (for MS + 2μM kinetin) up until the slowest treatment (EC) which took 244 days. The seeds on the MS + 128μM kinetin treatment were perhaps the best, taking 132 days to reach their maximum germination percentage of 100. Although the MS + 2μM kinetin treatment reached maximum germination faster, it only reached 89% germination. The majority of treatments reached maximum germination at 158 and 167 DAS (Fig. 2.4).

A number of seedlings died after germinating in 6 of the 8 treatments. Pearson's Chi-Squared test showed differences between treatments in respect to the percentage of seedlings that died ($\chi^2 = 29.81$; $p = 0.0001$). The only treatments in which all seedlings survived were VFT and MS + 2μM kinetin (Fig. 2.5) and as such, these treatments, especially VFT, were significantly different to the majority of other treatments (Table 2.14). The proportion of seedlings that died after germinating was quite low in most treatments, but was reasonably high in MS + 0.5μM kinetin, where 33% of the 100% of seeds that germinated later died (Fig. 2.5). The treatment with the lowest germination percentage, MS + 8μM kinetin, also had a high proportion of seedlings that died, with 33% of the 67% that germinated dying (Fig. 2.5). The two

treatments with the highest proportion of dead seedlings were significantly different to the majority of other treatments $p < 0.05$, Table 2.14).

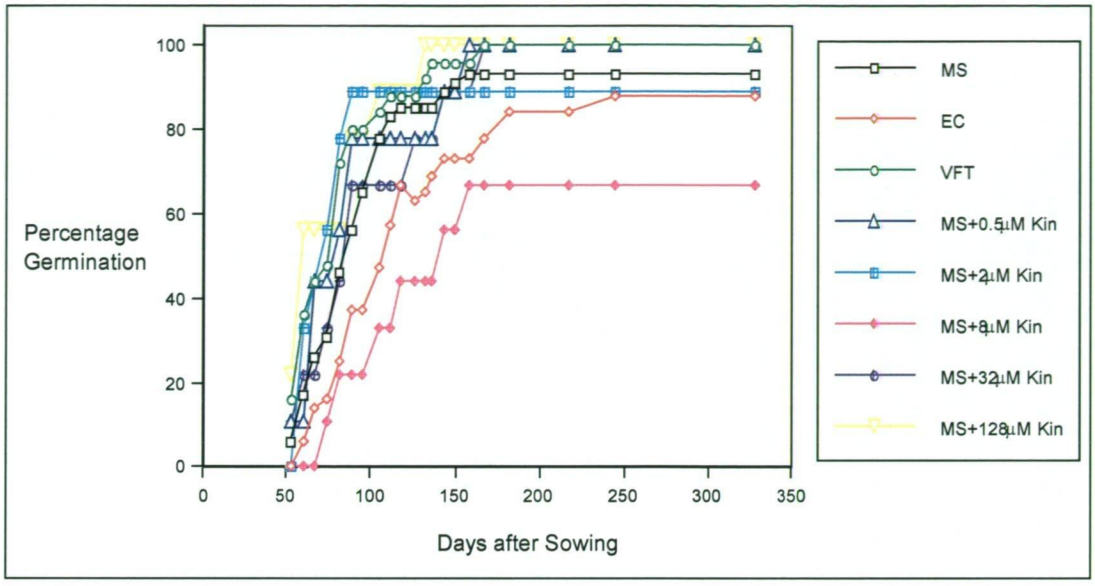

Figure 2.4. The percentage of *B. punicea* seeds that germinated *in vitro* on a range of media types at the days after sowing shown. Kin = kinetin.

Table 2.13. p-values for pairwise comparisons of percentage germination for *B. punicea* media treatments *in vitro*, using Chi-Squared tests. Significant values ($p < 0.05$) are highlighted.

	p-values						
Treatment	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	0.44	0.05	0.24	0.70	0.01	0.24	0.24
2		0.01	0.12	0.86	0.05	0.12	0.12
3			1.00	0.03	0.00	1.00	1.00
4				0.15	0.01	1.00	1.00
5					0.09	0.15	0.15
6						0.01	0.01
7							1.00

T1 = MS
T2 = EC
T3 = VFT
T4 = MS + 0.5µM kinetin
T5 = MS + 2µM kinetin
T6 = MS + 8µM kinetin
T7 = MS + 32µM kinetin
T8 = MS + 128µM kinetin

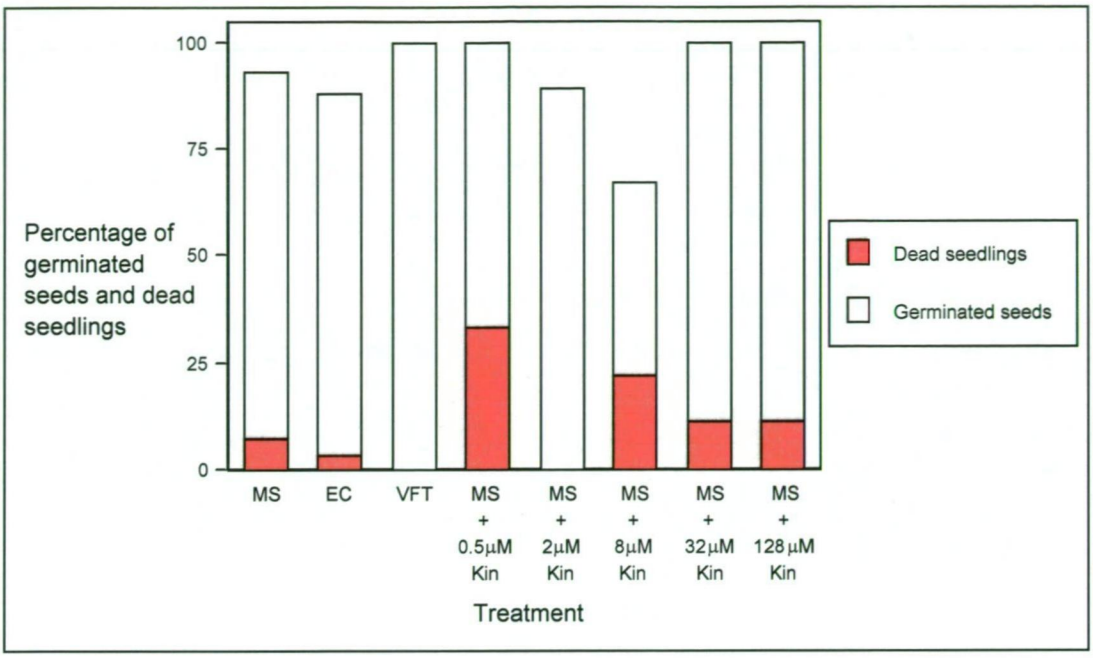

Figure 2.5. The percentage of *B. punicea* seeds that germinated and the proportion of seedlings that later died in each media treatment *in vitro*. Kin = kinetin.

Table 2.14. p-values for pairwise comparisons of percentage of dead seedlings for *B. punicea* media treatments *in vitro*, using Chi-Squared tests. Significant values ($p < 0.05$) are highlighted.

	p-values						
Treatment	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	0.50	0.04	0.01	0.23	0.02	0.75	0.75
2		0.14	0.00	0.38	0.00	0.37	0.37
3			0.00	1.00	0.00	0.03	0.03
4				0.01	1.00	0.09	0.09
5					0.01	0.17	0.17
6						0.11	0.11
7							1.00

T1 = MS
T2 = EC
T3 = VFT
T4 = MS + 0.5μM kinetin
T5 = MS + 2μM kinetin
T6 = MS + 8μM kinetin
T7 = MS + 32μM kinetin
T8 = MS + 128μM kinetin

2.3.1.2 *Dianella tasmanica*

2.3.1.2.1 *D. tasmanica* oven experiment

Seeds began to germinate 79 DAS. The first seeds to germinate were from the oven 50°C treatment (Fig. 2.6). These seeds germinated 35 days before the control seeds began to germinate (114 DAS). By the time the first control seed had germinated, 5 seeds (10%) had germinated in the oven 50°C treatment (Fig. 2.6).

The ANOVA provided evidence of statistical differences between treatments ($p = 0.0013$) (Table 2.15). There was no germination of any seeds from the oven 80°C or oven 100°C treatments, and as such, these treatments were significantly different to the control and oven 50°C treatments (Table 2.16). At the final assessment date, 534 DAS, exactly the same number of seeds had germinated in the control and oven 50°C treatments (Fig. 2.6). Therefore, an oven treatment at 50°C did not increase the overall germination percentage of *D. tasmanica* seeds ($p > 0.05$, Table 2.16). The percentage of germinated seeds was quite low for both of these treatments (38%).

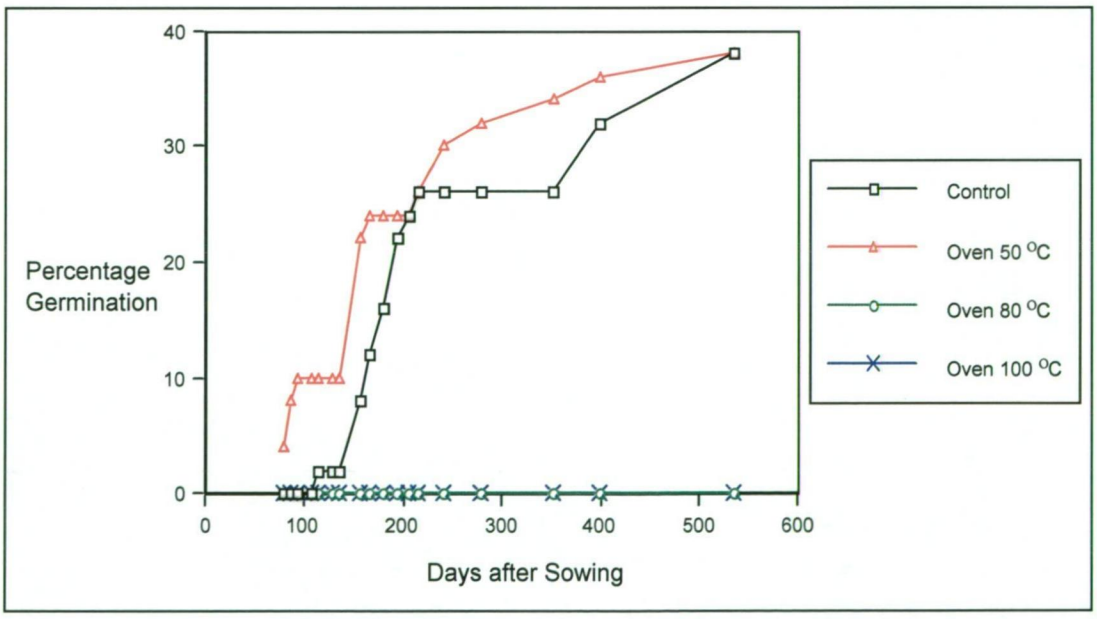

Figure 2.6. Percentage germination of *D. tasmanica* seeds in each oven treatment.

Table 2.15. Analysis of Variance for final germination data of *D. tasmanica* control and oven treated seeds.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-value
MAIN EFFECTS					
A: Treatment	72.2	3	24.0667	10.20	0.0013
B: Replicate	21.3	4	5.325	2.26	0.1234
RESIDUAL	28.3	12	2.35833		
TOTAL (CORRECTED)	121.8	19			

Table 2.16. Tukey's HSD test groupings of oven treated *D. tasmanica* seeds (Method: 95.0 percent HSD). Values followed by the same letter are not significantly different (p = 0.05).

Treatment	Mean
1. Control	3.8 b
2. Oven 50°C	3.8 b
3. Oven 80°C	0.0 a
4. Oven 100°C	0.0 a

2.3.1.2.2 *D. tasmanica* fermentation experiment

Seeds began to germinate first in the control treatment, 125 DAS. Seeds in the fermented treatment did not start to germinate until 169 days after the control seed (294 DAS) (Fig. 2.7). The rate of germination was quite slow in both treatments until after approximately 351 DAS when there was quite a rapid increase in a 30 day period (from 6.7% to 18.9% for control seeds and from 8.3% to 33.9% for fermented seeds). At the final assessment date only 69 of the 180 control seeds had germinated (38.3%), while 90 fermented seeds (50%) had germinated. However, the ANOVA showed that fermentation of *D. tasmanica* seeds did not significantly increase germination compared to the control (p = 0.1376) (Table 2.17).

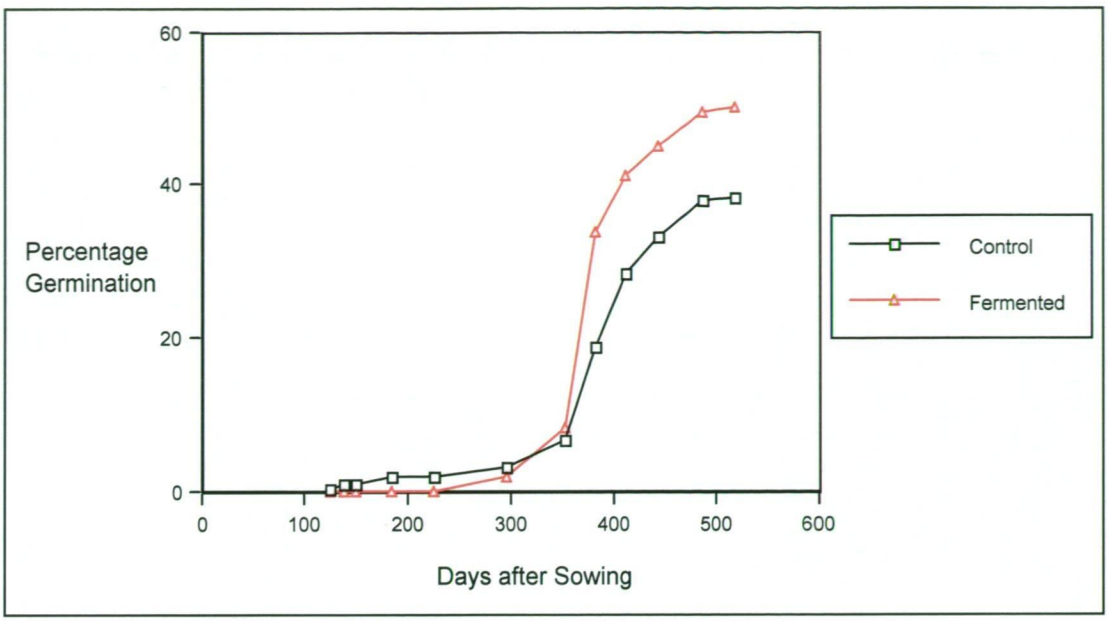

Figure 2.7. Percentage germination of *D. tasmanica* seeds in the control and fermented treatments at each recording date.

Table 2.17. Analysis of Variance for final germination data of *D. tasmanica* control and fermented seeds.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-value
MAIN EFFECTS					
A: Treatment	24.5	1	24.5	2.72	0.1376
B: Replicate	139.111	8	17.3889	1.93	0.1854
RESIDUAL	0	8	9.0		
TOTAL (CORRECTED)	235.611	17			

2.3.1.2.3 *D. tasmanica* smoke experiment

D. tasmanica seeds from the direct smoke treatment began to germinate 65 DAS (Fig. 2.8). With the exception of the 100% smoke treatment, which was the slowest treatment to begin germinating (128 DAS), all other smoke treated seeds began to germinate before the control seeds, which did not begin to germinate until 121 DAS (50% smoke - 72 days, 10% smoke - 86 days and 1% smoke - 114 days) (Fig. 2.8).

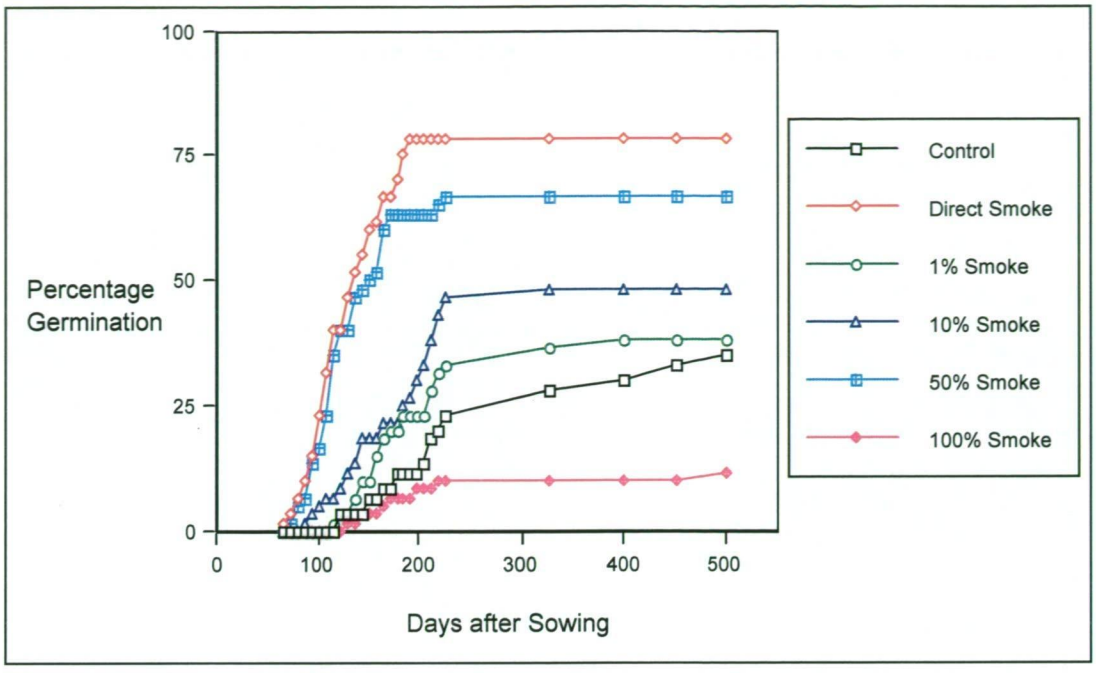

Figure 2.8. Percentage germination of *D. tasmanica* seeds in each smoke treatment.

The ANOVA showed that there were differences between treatment means in regard to germination ($p = 0.00$; Table 2.18). All of the smoke treatments increased the percentage germination of *D. tasmanica* seeds, compared to the control, with the exception of 100% smoke, which significantly inhibited germination (Fig. 2.8; Table 2.19). 1% smoke and 10% smoke did not significantly increase the mean germination compared to the control (Table 2.19). The best germination percentage (78.3%) occurred when seeds were treated with direct smoke (Fig. 2.8). Smoke extract solutions were also effective, with 50% smoke producing the best result (66.7%). Tukey's HSD test showed that the direct smoke and 50% smoke treatments were not significantly different to each other, but were significantly different to all other treatments, with the exception of 10% smoke (Table 2.19). There was a trend of increasing percentage germination with increasing smoke extract concentration, up to, and including, 50% smoke. As mentioned above, the undiluted (100%) smoke extract solution significantly inhibited germination, compared to the control (Fig. 2.8; Table 2.19).

D. tasmanica seeds took between 191 and 500+ days to reach full germination, with the germination capacity reached more quickly in smoke treated seeds (with the

exception of 100% smoke) than the control (Fig. 2.8). Direct smoke treated seeds reached maximum germination fastest (191 DAS), while the slowest treatments (control and 100% smoke) still had seeds germinating 500 DAS (Fig. 2.8). Seeds from the 50% smoke extract treatment reached maximum germination 226 DAS, which was the fastest result for all smoke extract treatments (Fig. 2.8). The 50% smoke treated seeds reached maximum germination 100 days before the 10% smoke seeds (326 DAS) and 174 days before the 1% smoke seeds (400 DAS) (Fig. 2.8).

Table 2.18. Analysis of Variance for final germination data of *D. tasmanica* control and smoke treated seeds.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-value
MAIN EFFECTS					
A: Treatment	170.139	5	34.028	23.7919	0.000
B: Replicate	10.688	1	10.688	7.473	0.011
RESIDUAL	41.479	29	1.430		
TOTAL (CORRECTED)	222.306	35			

Table 2.19. Tukey's HSD test groupings of smoke treated *D. tasmanica* seeds (Method: 95.0 percent HSD). Values followed by the same letter are not significantly different ($p = 0.05$).

Treatment	Mean
1. Control	3.5 b
2. Direct smoke	7.83 d
3. 1% smoke	3.83 b
4. 10% smoke	4.83 bc
5. 50% smoke	6.67 cd
6. 100% smoke	1.17 a

2.3.1.2.4 *D. tasmanica* partial removal of testa experiment

Pearson's Chi-Squared test showed differences between treatments in respect of percentage germination ($\chi^2 = 49.1$, $p = 0.00$).

Control seeds failed to germinate, while 90% of the seeds that had part of their testa removed, germinated. The partial testa removal treated seeds began to germinate within 12 days in culture, with maximum germination (90%) reached within 32 days

(Plate 2.5). There was no further germination from seeds in either treatment (seeds were monitored for a total of 90 days).

Plate 2.5. *D. tasmanica* seedling that has germinated *in vitro*, after part of the testa was removed. Scale bar = 1 cm.

2.3.1.3 *Milligania densiflora*

2.3.1.3.1 Initial *M. densiflora* seed germination experiment

Pearson's Chi-Squared test showed differences between treatments in respect of percentage germination ($\chi^2 = 31.1$, $p = 0.00$).

Seeds from the MBG failed to germinate, regardless of their location in the incubation room or in the laboratory (Fig. 2.9), and as such the percentage germination of the MBG treatments was significantly different to both Mt Read seed treatments ($p = < 0.05$) (Table 2.20). However, the fresher seeds collected from Mt Read germinated in both locations, with seeds in the incubation room reaching a significantly higher germination percentage than those in the laboratory ($p = 0.01$) (Fig. 2.9; Table 2.20).

Seeds from both the laboratory and the incubation room began to germinate approximately 17 DAS, however the rate of germination was more rapid in the seeds in the incubation room in the early stages of the experiment, with 45.5% germinated

43 DAS, compared to only 4.8% of the seeds grown in the laboratory (Fig. 2.9). At the final recording date (146 DAS) the seeds in the laboratory appeared to have nearly reached their full germination capacity (Fig. 2.9), while those in the incubation room were still germinating at a steady rate. Unfortunately, the seeds in the incubation room were overtaken by a fungal contaminant, so the experiment was terminated at this point.

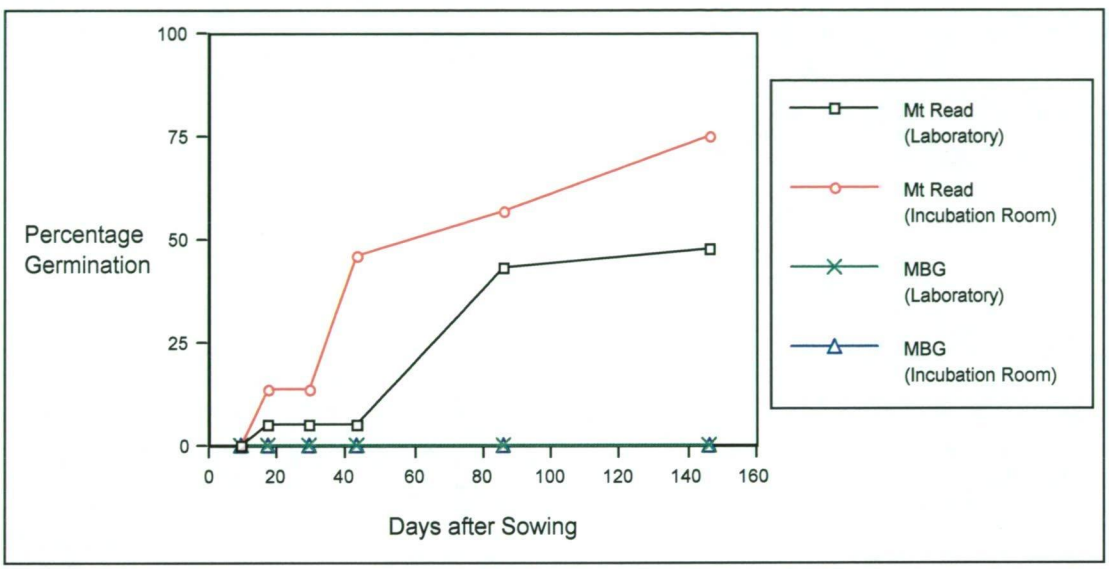

Figure 2.9. Percentage germination of *M. densiflora* seeds from Mount Read (Tasmania) and Melbourne Botanic Gardens (MBG) in the laboratory and the incubation room.

Table 2.20. p-values for pairwise comparisons of percentage germination for *M. densiflora* seeds, from MBG and Mt Read, grown in the laboratory and the incubation room using Chi-Squared tests. Significant values ($p < 0.05$) are highlighted.

	p-values		
Treatment	**2**	**3**	**4**
1	1.00	0.00	0.01
2		0.00	0.01
3			0.01

T1 = MBG (Incubation Room)
T2 = MBG (Laboratory)
T3 = Mt Read (Incubation Room)
T4 = Mt Read (Laboratory)

2.3.1.3.2 Seed germination of *M. densiflora in vitro*

Before seeds began to germinate, some became contaminated. Nine days after placement on the media, 62% (or 39) of the Mt Read seeds were contaminated, but those from the MBG were still clean. A further 8 days later, 5 more seeds from Mt Read (70% total) were contaminated, while 4 from the MBG were also contaminated (17%). It was at this time (17 DAS) that the first seed germinated, at the same time as seeds began to germinate in petri dishes in the experiment above (Fig. 2.10). By the time the experiment finished (146 DAS), 75% of the Mt Read seeds were contaminated, and 62.5% of those from the MBG.

As with the previous experiment, none of the seeds from the MBG germinated, and only 22% from Mt Read (including contaminated seed). However, if the contaminated seed is not included, 88% of the remaining seeds germinated. Of the seed that did germinate, only 50% survived, the other half died soon after germinating.

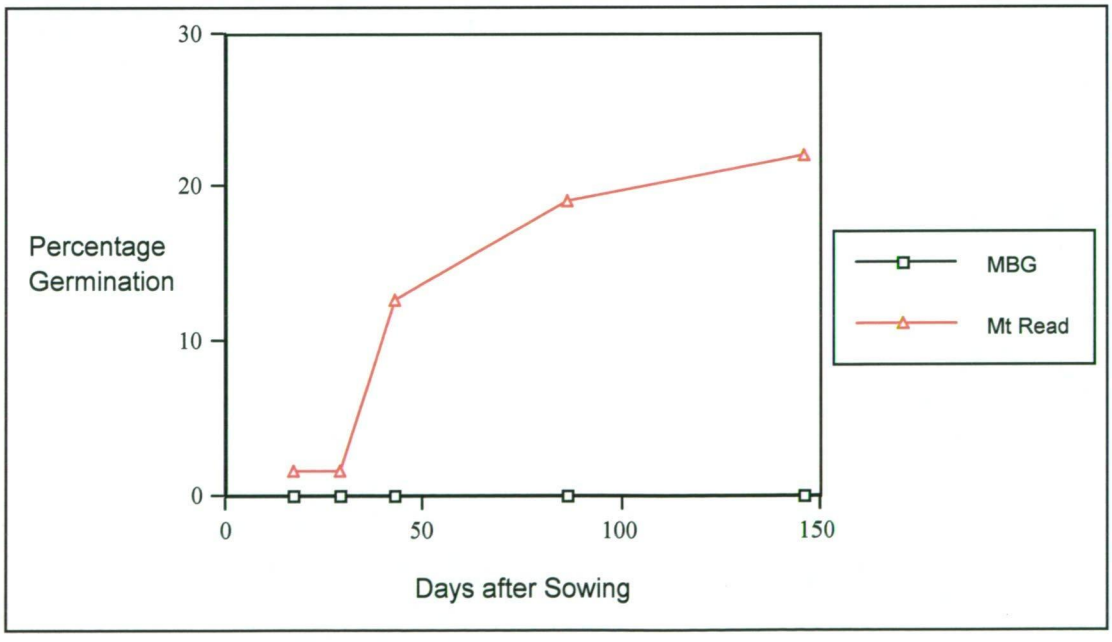

Figure 2.10. Percentage germination of *M. densiflora* seeds from Mount Read (Tasmania) and MBG *in vitro*.

2.3.1.3.3 Seed germination of *M. densiflora in vitro* when disinfested for different times and placed on two media types

2.3.1.3.3.1 Contamination

Pearson's Chi-Squared test showed differences between treatments in respect to the percentage of seeds that became contaminated ($\chi^2 = 18.2$, $p = 0.00$).

The percentage of seed contaminated was significantly higher for both media types when seeds were disinfested for 20 min, compared to 35 min (Table 2.21) ($p < 0.05$ for all pairwise comparisons between 20 min and 35 min treatments; see Table 2.22). The best treatment, with the lowest percentage of contaminated seeds was MS (35 min) where only 17% of seeds were contaminated (Table 2.21), but this was not significantly different to the 1/2 MS (35 min) treatment (Table 2.22). Most of the contamination occurred within 16 days of placement on the media (regardless of media type), with only a few more seeds becoming contaminated later in the experiment.

Table 2.21. The percentage of *M. densiflora* seeds that were contaminated at each recording date for each media and disinfestation treatment.

	Percentage of seeds contaminated (days after sowing)			
Treatment	**0**	**16**	**45**	**165**
1. MS (20 min)	0	45	47	48
2. MS (35 min)	0	12	13	17
3. 1/2 MS (20 min)	0	40	40	43
4. 1/2 MS (35 min)	0	15	20	25

Table 2.22. p-values for pairwise comparisons of percentage contamination for *M. densiflora* seeds disinfested for 20 or 35 min and grown on MS or 1/2 MS media using Chi-Squared tests. Significant values ($p < 0.05$) are highlighted. Treatments are listed in Table 2.21.

	p-values		
Treatment	**2**	**3**	**4**
1	0.00	0.58	0.01
2		0.00	0.26
3			0.03

2.3.1.3.3.2 Germination

Seed began to germinate 16 days after placement on the media (Fig. 2.11) in all treatments. To avoid the confounding effect of contamination, the germination of seeds has been expressed as a percentage of the uncontaminated seeds only, and not the total number of seeds. Pearson's Chi-Squared test showed differences between treatments in respect to percentage germination ($\chi^2 = 9.8$, $p = 0.02$). The best germination (90%) occurred in the treatment where seeds were disinfested for 35 min and then grown on 1/2 MS (Fig. 2.11), and this was the only treatment that was significantly different to the other treatments ($p < 0.05$, see Table 2.23). Interestingly, the seeds disinfested for only 20 min had a lower germination percentage when grown on both media types, compared to those that were treated for 35 min (Fig. 2.11). However, this was only significantly different in the pairwise comparison between the two treatments on 1/2 MS media (Table 2.23).

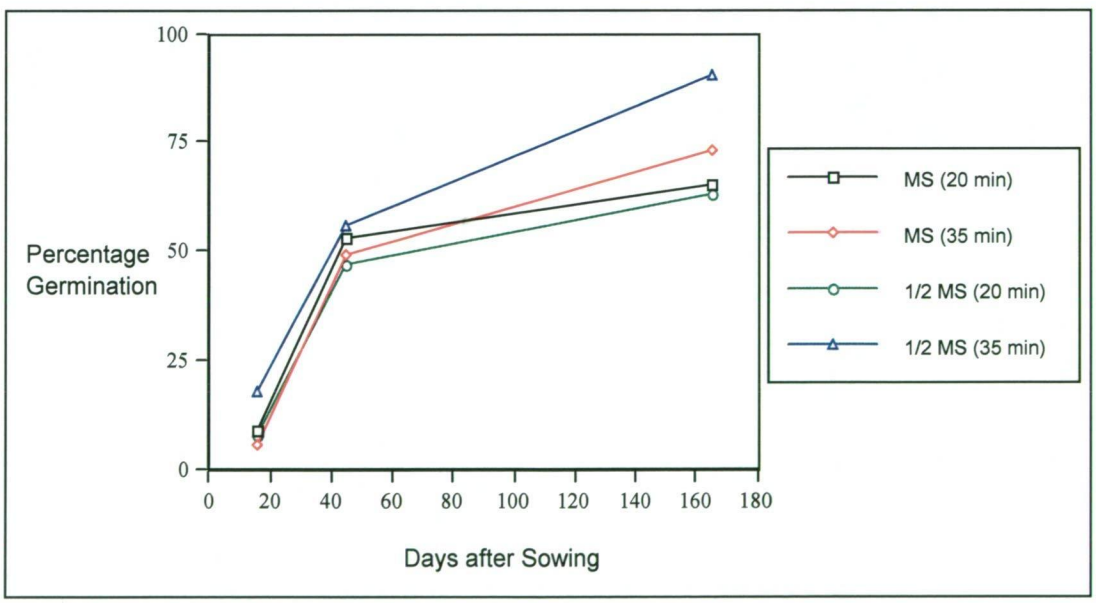

Figure 2.11. Percentage germination of uncontaminated *M. densiflora seeds in vitro* on two different media concentrations and with different disinfestation times.

Table 2.23. p-values for pairwise comparisons of percentage germination for *M. densiflora* seeds disinfested for 20 or 35 min and grown on MS or 1/2 MS media using Chi-Squared tests. Significant values ($p < 0.05$) are highlighted. Treatments are listed in Table 2.21.

	p-values		
Treatment	**2**	**3**	**4**
1	0.45	0.89	0.01
2		0.35	0.03
3			0.00

2.3.1.3.3.3 Seedling survival

A number of seedlings died after germinating in all treatments. Pearson's Chi-Squared test showed differences between treatments in respect to the percentage of seedlings that died ($\chi^2 = 14.9$, $p = 0.00$).

Seedlings survived better on the 1/2 MS media than those growing on the full-strength MS media (Fig. 2.12). Seeds disinfested for 20 min and grown on 1/2 MS survived best, with only 8.8% of the 63% of seeds that germinated, later dying (Fig. 2.12). This treatment was the only one that was significantly different to all other treatments ($p < 0.01$; Table 2.24).

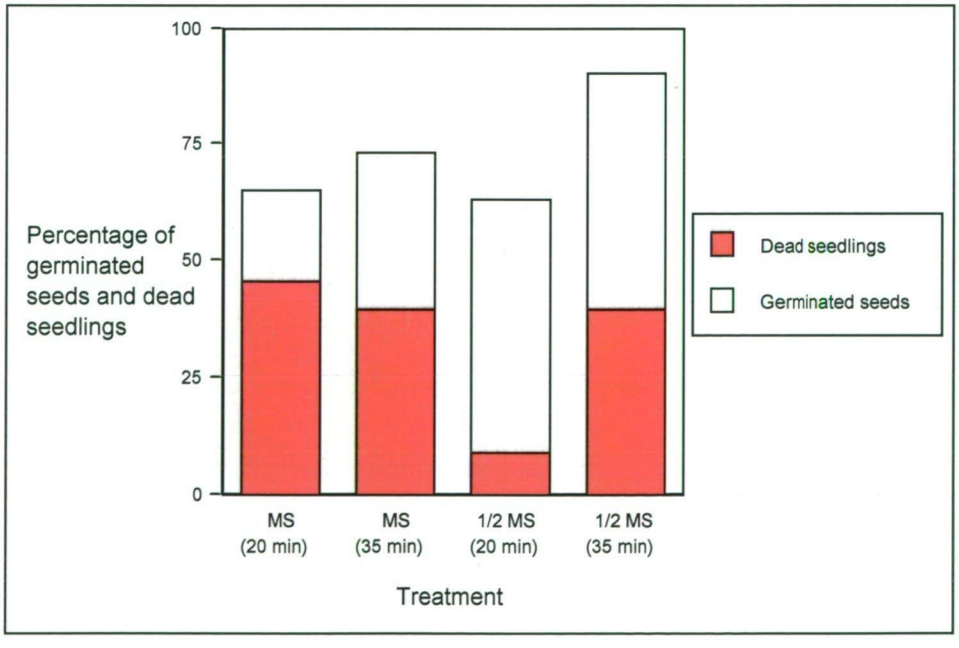

Figure 2.12. The percentage of *M. densiflora* seeds that germinated and the proportion of seedlings that later died on each media treatment and disinfestation period *in vitro*.

Table 2.24. p-values for pairwise comparisons of percentage of dead seedlings for *M. densiflora* seeds disinfested for 20 or 35 min and grown on MS or 1/2 MS media, using Chi-Squared tests. Significant values ($p < 0.05$) are highlighted. Treatments are listed in Table 2.21.

	p-values		
Treatment	**2**	**3**	**4**
1	0.24	0.00	0.06
2		0.00	0.38
3			0.01

2.3.2 Iridaceae Seed Germination Experiments

2.3.2.1 *Diplarrena moraea*

2.3.2.1.1 *D. moraea* initial *in vitro* seed germination experiment

Only one seed germinated from the 24 seeds that were initially placed *in vitro*. This seed was from the group disinfested in 2% NaOCl, and took five days to germinate. By the time this seedling was 1.5 months old, it had three leaves, but the ends of the leaves had lost their colour and did not look healthy. The experiment was concluded approximately seven months later, as no further germination had taken place.

Contamination was a problem, especially with the seeds that were disinfested in the weaker 1% NaOCl solution. Six days after seeds were placed on the MS medium only 2 of the 12 seeds given this treatment were not contaminated. However, 8 of the 12 treated with 2% NaOCl were contaminant free.

2.3.2.1.2 *D. moraea* second *in vitro* seed germination experiment

The second *D. moraea* seed germination experiment was much more successful than the first. The disinfestation procedure was 100% successful and 97% of the seeds had germinated by the time the experiment was concluded (approximately 8 months after the experiment began). Seeds began to germinate 10 days after they were placed on the medium (Fig. 2.13). There was a steady increase in the germination

rate, and the majority (88%) of seeds had germinated 51 days after placement on the media.

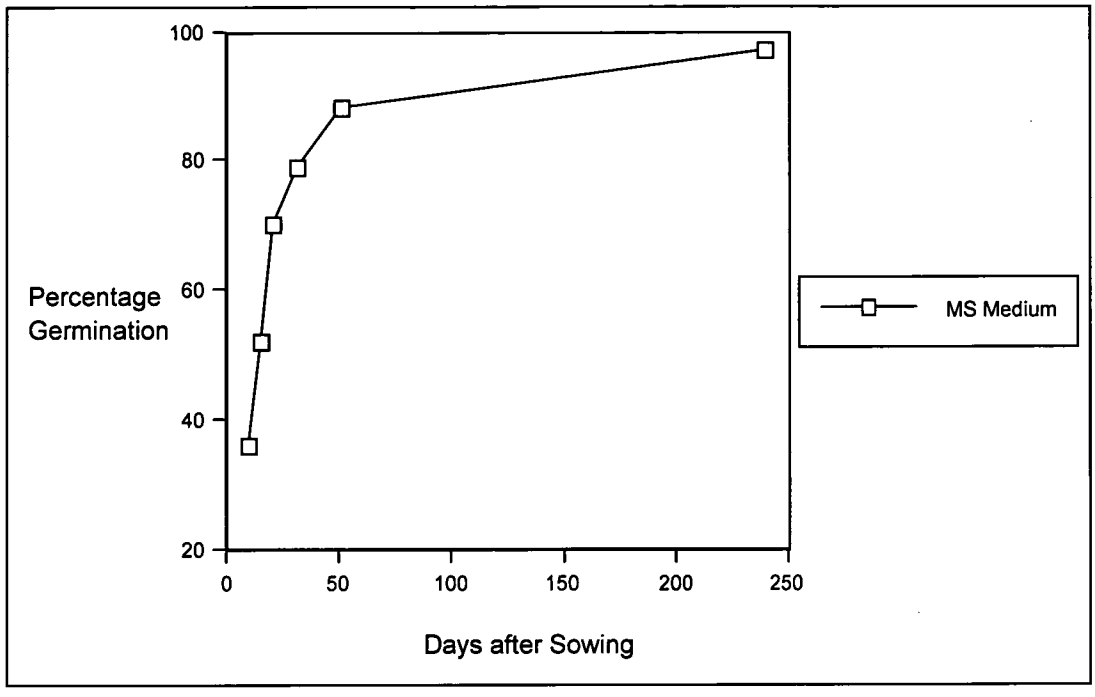

Figure 2.13. The percentage germination of *D. moraea* seeds grown on MS media *in vitro*.

2.3.2.2 *Diplarrena latifolia*

2.3.2.2.1 *D. latifolia in vitro* media type and kinetin experiment

D. latifolia seeds began to germinate 10 days after placement on the media in all treatments grown in the light (Figs 2.14, 2.15). Those in the dark took slightly longer; not beginning to germinate until a further 15 days (25 days after placement on the media) (Fig. 2.14). Pearson's Chi-Squared statistic showed differences between treatments in respect of percentage germination ($\chi^2 = 29.7$, $p = 0.00$). Seeds on the MS medium reached a higher germination percentage than those on the EC medium, regardless of whether they were grown in the light or dark. However, this difference was only significant in pairwise comparisons between MS light and the EC treatments ($p < 0.05$, see Table 2.25). On both these media, seeds germinated slightly better in the light than the dark (Fig. 2.14; n.s., see Table 2.25).

Table 2.25. p-values for pairwise comparisons of percentage of germinated seeds for *D. latifolia* media treatments *in vitro*, using Chi-Squared Tests. Significant values ($p < 0.05$) are highlighted.

	p-values							
Treatment	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	0.48	0.04	0.02	0.00	0.61	0.22	0.31	0.24
2		0.20	0.12	0.01	0.89	0.08	0.75	0.09
3			0.78	0.17	0.17	0.00	0.39	0.01
4				0.29	0.11	0.00	0.27	0.00
5					0.01	0.00	0.04	0.00
6						0.11	0.63	0.13
7							0.05	0.92
8								0.06

T1 = MS Light
T2 = MS Dark
T3 = EC Light
T4 = EC Dark
T5 = MS + 0.5μM kinetin
T6 = MS + 2μM kinetin
T7 = MS + 8μM kinetin
T8 = MS + 32μM kinetin
T9 = MS + 128μM kinetin

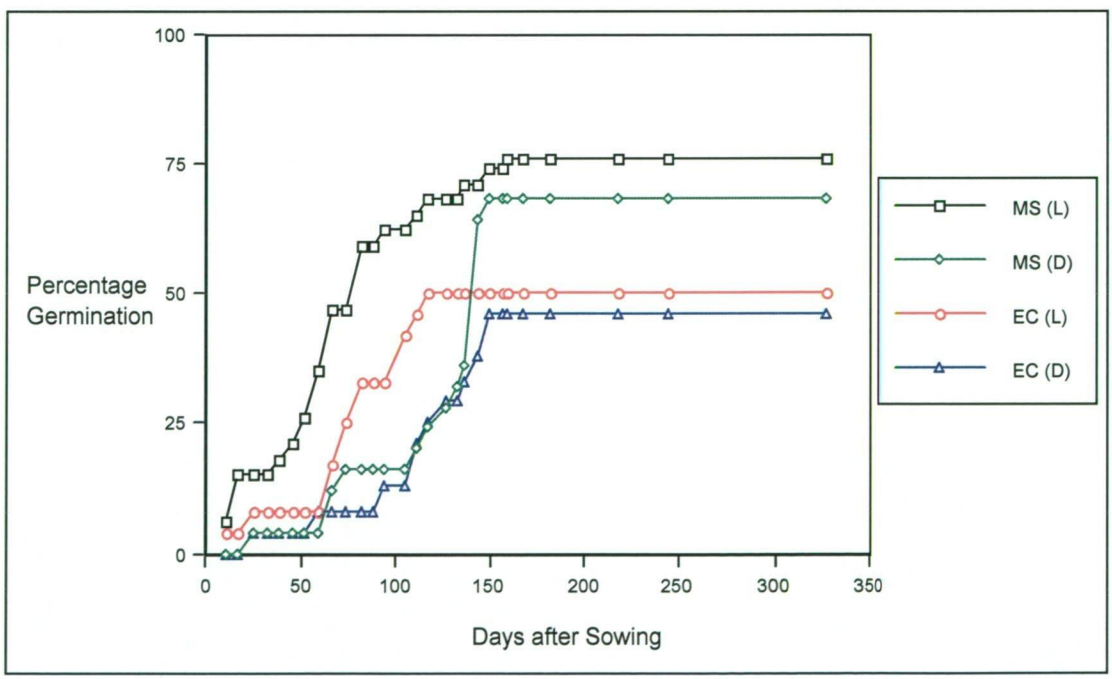

Figure 2.14. The germination percentages of *D. latifolia* seeds *in vitro* when grown on MS (control) and EC media under light and dark conditions. L = light, D = dark.

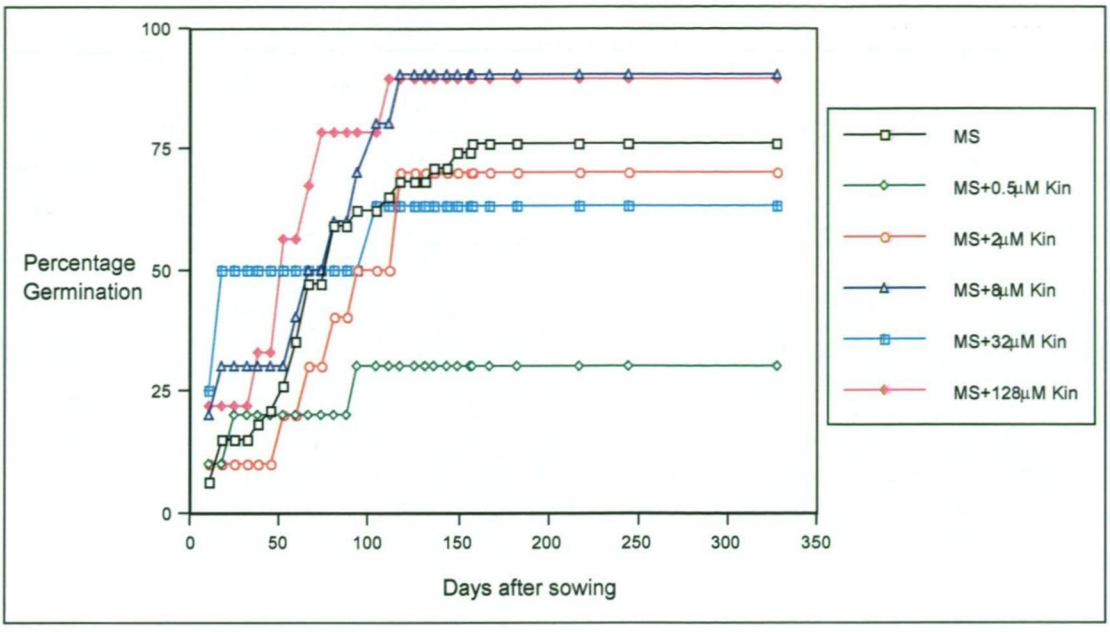

Figure 2.15. The germination percentages of *D. latifolia* seeds *in vitro* when grown on MS media (control) or MS supplemented with different concentrations of kinetin (Kin).

With the exception of MS + 32µM kinetin, a high concentration (> 8µM) of kinetin in the media increased the germination of seeds, when compared to the control (MS light), but was not significantly different (Table 2.25). The best germination was achieved by seeds on the MS + 8µM kinetin treatment, where 90% germinated (Fig. 2.15). However, this germination percentage was only significantly different to the EC light, EC dark and MS + 0.5µM kinetin treatments ($p < 0.05$; see Table 2.25). MS + 128µM kinetin also produced excellent germination (88.9%), and was also only significantly different to the same three treatments ($p < 0.05$; Table 2.25). The lowest germination percentage occurred in the treatment containing MS + 0.5µM kinetin, where only 30% of seeds germinated, and as such this treatment was significantly different to the majority of other treatments, with the exception of EC light and EC dark (Table 2.25).

In all treatments, except for MS + 2µM kinetin, some of the seeds that germinated later died. The proportion of dead seedlings varied with treatment (Fig. 2.16), with the worst performing treatment, in terms of germination percentage (MS + 0.5µM kinetin) also containing the highest proportion of dead seedlings (only 10% of seedlings survived). The MS light treatment also had a fairly high proportion of dead

seedlings, with more than one third of the seeds that germinated not surviving. In general, apart from the aforementioned treatments, the proportion of dead seedlings was quite low, usually under 20%. Pearson's Chi-Squared statistic showed that there were no significant differences between treatments in respect to percentage of dead seedlings ($\chi^2 = 15.3$, $p= 0.05$). When the proportion of dead seedlings is taken into account, the best treatment is probably MS + 128μM kinetin, where only 12.5% of the 88.9% of seeds that germinated later died. The MS + 8μM kinetin treatment still performed well, but is reduced to the same percentage germination as the MS + 2μM kinetin treatment, when the proportion of dead seedlings is taken into account.

In regard to the time to germination, the presence of kinetin in the medium appeared to speed up the germination process, particularly at the higher concentrations. Ten DAS, greater than 20% of seeds had germinated in the treatments supplemented with the three highest concentrations of kinetin (Fig. 2.15). Germination in the MS + 32μM kinetin treatment was particularly fast, with 50% of seeds germinated 17 DAS (Fig. 2.15). The time taken to reach germination capacity (the maximum germination achieved) varied between 94 to 158 DAS, with the worst treatment in terms of germination percentage (MS + 0.5μM kinetin), being the quickest to reach germination capacity (Fig. 2.15). The slowest was the control (MS light) treatment. The treatments with the highest germination percentages reached their germination capacities quite early within the range, at 111 and 117 DAS for MS + 128μM kinetin and MS + 8μM kinetin, respectively.

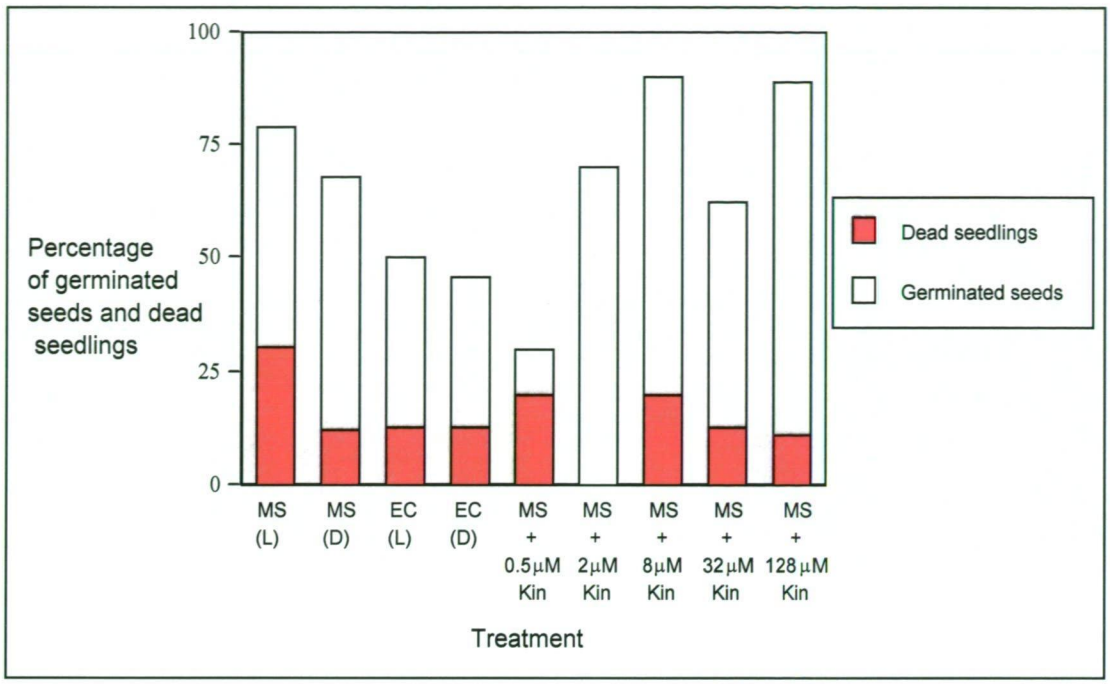

Figure 2.16. The percentage of *D. latifolia* seeds that germinated, and the proportion of seedlings that later died in each treatment *in vitro*. Kin = kinetin.

2.3.2.3 *Isophysis tasmanica*

2.3.2.3.1 *I. tasmanica* light/dark experiment *in vitro*

The disinfestation protocol (2% NaOCl for 15 min) was successful, with 100% of seeds remaining contaminant-free for the duration of the experiment.

Seeds began to germinate 48 DAS, and the first seeds to germinate were in dark conditions (Fig. 2.17). The seeds in the light took longer to germinate, 60% of seeds in the dark had germinated by the time the first seed in the light had begun to germinate (Fig. 2.17).

The experiment was concluded approximately 4 months after it began as no further germination was occurring. Percentage germination of *I. tasmanica* seeds was significantly higher in the dark (100%) than the light (40%) ($\chi^2 = 17.14$, $p = 0.00$). In addition, the seedlings from the light treatment all looked unhealthy and appeared to be dying, while those from the dark treatment were still green and healthy (approximately one week after they had germinated the dark treatment seeds were removed from the box and placed in the same conditions as the light treated seeds).

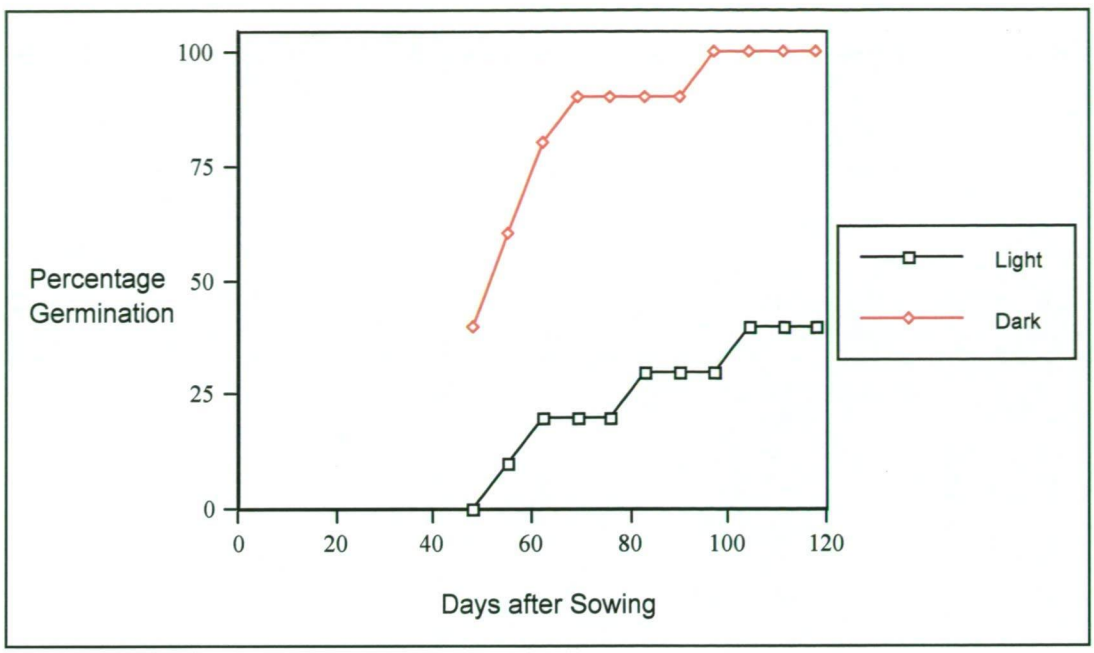

Figure 2.17. Percentage germination of *I. tasmanica* seeds grown in light and dark conditions *in vitro*.

2.3.2.3.2 *I. tasmanica* smoke solution experiment

I. tasmanica seeds began to germinate 54 DAS in the majority of treatments (Fig. 2.18). With the exception of the 100% smoke dark treatment, seeds germinated more rapidly when placed in the dark, for example, 40% of the control dark seeds had germinated, compared to 0% of the control light seeds 54 DAS (Fig. 2.18). The ANOVA suggested that there were no differences between treatment means in regard to germination (Table 2.26). Therefore a smoke treatment does not significantly increase germination percentages of *I. tasmanica*. 100% germination occurred in 4 of the 8 treatments. With the exception of the 50% smoke light treatment, these were all dark treatments (Fig. 2.18). The control light and 10% smoke light treatment also produced excellent germination, with 90% of seeds germinating. The 100% smoke treatment appeared to inhibit germination, and this was more pronounced in the dark treatment, where only 45% of seeds germinated (n.s.).

Seeds took between 94 and 173 days to reach full germination, with the germination capacity generally being reached more quickly in the dark treatments (Fig. 2.18). Seeds germinated fastest in the control dark, 10% smoke dark and 50% smoke dark treatments. At the time these treatments had reached 100% germination (94 DAS),

their counterparts under light conditions had only reached 55%, 55% and 75%, respectively (Fig. 2.18).

It should be noted that fungal contaminants were a problem in the 100% smoke dark treatment, with a total of 10 seeds infected 94 DAS. However, one mouldy seed did later germinate.

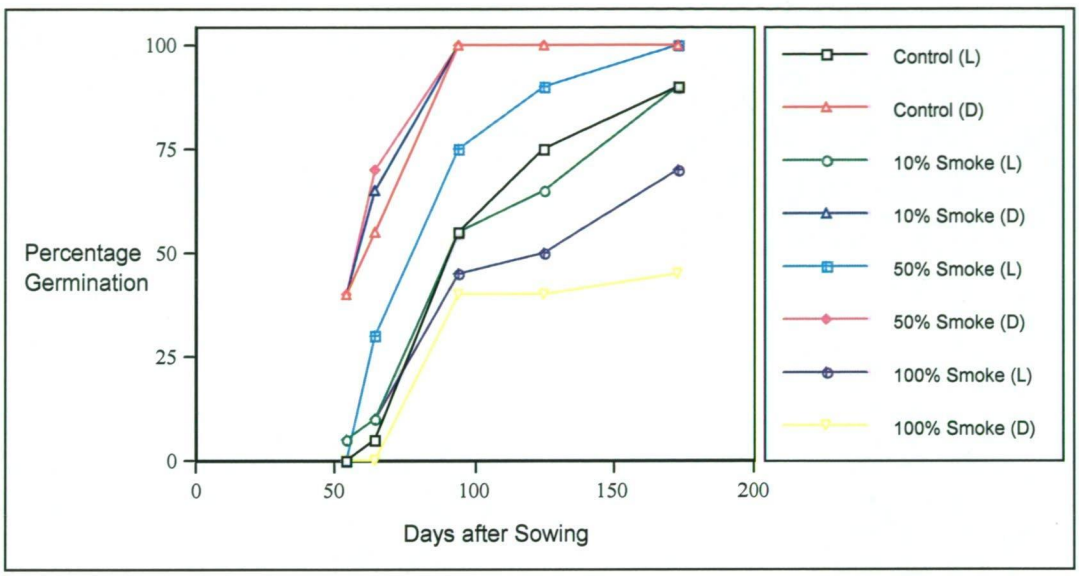

Figure 2.18. The percentage of *I. tasmanica* seeds that germinated in each smoke treatment, in light (L) and dark (D) conditions.

Table 2.26. Analysis of Variance for final germination data of *I. tasmanica* control and smoke solution treated seeds.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-value
MAIN EFFECTS					
A: Treatment	54.9375	7	7.84821	2.45	0.130
B: Replicate	0.0625	1	0.0625	0.02	0.893
RESIDUAL	22.4375	7	3.20536		
TOTAL (CORRECTED)	77.4375	15			

2.4 Discussion

Propagation by seed is indeed possible for all of the species included in this study, with germination occurring in all experiments.

With the exception of *Dianella tasmanica*, the natural germination percentages of all species studied was generally quite high. *Blandfordia punicea* (Liliaceae) and *Isophysis tasmanica* (Iridaceae) had the highest natural levels. Without any seed pre-treatment, *B. punicea* seeds consistently reached greater than 93% germination both *in vitro* and in petri dishes, with 100% being reached by control treatments in two of the three experiments performed. *I. tasmanica* seeds reached 90% germination in the light and 100% in the dark, in petri dishes. The two *Diplarrena* species had fairly high germination percentages *in vitro* - *D. moraea* reaching 97% and *D. latifolia* 76% on MS medium. *Milligania densiflora* was inconsistent, reaching only 48% germination in petri dishes, but 88% on MS medium *in vitro*. Without any seed pre-treatment the germination of *Dianella tasmanica* was low, but consistent, with only 38% germination in both experiments. This was also the case in previous experiments with this species, where the natural germination levels reached a maximum of 30% (Sward, 1995).

2.4.1 Liliaceae Seed Germination Experiments

2.4.1.1 *Blandfordia punicea*

2.4.1.1.1 *B. punicea* smoke solution experiment

Promotion of germination by smoke and smoke extracts has been a widely studied phenomenon throughout fire-prone regions of the world. This relatively new technology has huge implications for the horticulture and floriculture industries as it has allowed the propagation of many species (some of which have potential for these industries), that have previously been difficult or impossible using conventional methods. In Australia a wide range of species have been studied, however, only a few studies have included native Liliaceae and Iridaceae species (see Bell *et al.*,

1987; Dixon and Roche, 1995; Dixon *et al.*, 1995; Hopkins *et al.*, 2000; Tieu *et al.*, 2001; Allan *et al.*, 2004). Within Tasmania research has mainly focused on heath species, especially from the Epacridaceae family (see Keith, 1997; Gilmour *et al.*, 2000). However, preliminary Tasmanian research has shown that *Dianella tasmanica* (Liliaceae) is responsive to smoke (Sward, 1995), and it was thought relevant to test the effects of a smoke solution on seed germination of *Blandfordia punicea,* in both mature and immature seeds.

In this experiment seeds germinated from only two of the three seed lots used. The SC2 seed did not germinate at all, which was interesting as they were thought to be more mature than the SC3 seeds (which did germinate). Perhaps they were actually less mature than the SC3 seeds, or there may have been some kind of viability problem with this seed lot. The latter explanation is probably the most likely as the appearance of the seed was such that it was definitely thought to be more mature than SC3 (they were brown, compared to the green - light brown colour of the SC3 seeds).

The fact that SC1 seeds had a higher germination percentage than SC3 in all treatments is not surprising, as the SC1 seeds were mature and therefore at the correct stage of development for germination to take place. This shows that for optimum germination results, seed should be fully mature when collected.

The mature seed lot (SC1) had very high germination percentages in all treatments, with the control (light) treatment, amongst others, reaching 100%, which supports previous findings that no seed pre-treatments are required for this genus (Johnson, 1996a; Stewart and Stewart, 1999). Seed germinated equally as well in the light or dark and none of the smoke solutions either significantly enhanced or inhibited germination. Interestingly, when the seed was immature (SC3), the control seeds germinated better under dark conditions than light. This differing response to darkness shown between mature and immature seeds is not unexpected as the effects of light may vary in mature seeds according to factors such as temperature and storage (Mayer and Poljakoff-Mayber, 1975; Hartmann and Kester, 1983), and therefore are quite likely to also vary due to seed age. Also, the low to mid concentrations (10% and 50%) of smoke solution actually increased germination (but not significantly) when compared to the control (light) seeds, but not when compared

to the control (dark) seeds. Perhaps light has some kind of inhibitory effect on seeds which prevents them from germinating until they are fully mature, but this inhibition can be partially overcome by the presence of smoke in low to mid concentrations. The undiluted smoke (100% solution) significantly inhibited germination when the seeds were immature. Inhibition of germination by higher concentrations of smoked water has also been noted by other authors for a range of different species (Brown, 1993a; Baxter *et al.*, 1994; Brown *et al.*, 1994a; Dixon and Roche, 1995; Dixon *et al.*, 1995; Drewes *et al.*, 1995; Roche *et al.*, 1997a; Gilmour *et al.*, 2000). Under light conditions germination was completely suppressed. However, this inhibitory effect was slightly reduced when seeds were placed in the dark. Again, this suggests that light has some kind of inhibitory effect on germination of immature seeds. Further stressing this inhibitory effect of light on germination of immature seeds was the fact that the control seeds germinated better in the dark than in the light (although the difference was not significant).

Although the difference was only slight, it is not surprising that the more mature SC1 seeds began to germinate before the immature SC3 seeds, as they are at the correct stage of development for germination to occur. However, it was surprising that the percentage of seeds that had germinated (at the time SC3 seeds began to germinate) was actually higher for SC3 seeds, in the majority of treatments, than SC1. This shows that the seeds that did germinate from the SC3 seed lot, did so quite rapidly and then reached a maximum level that was much lower than those reached by the mature SC1 seeds. The same pattern of quite a rapid rate of germination as soon as germination had commenced was also noticed in the SC1 seed lot in all treatments. Therefore, under natural conditions, all (or at least the majority of) seedlings would be growing at the same time, leaving them vulnerable to an unforseen event such as an out of season frost or a fire, which could kill all of the seedlings from one plant at once. Perhaps this is why *Blandfordia* are such prolific seeders (Johnson, 1996a). The loss of an entire season's seedlings is also not necessarily a significant one for this genus as it can also reproduce vegetatively from the rhizomatous corms.

The times taken to germinate in this experiment are slightly longer than the 14 - 21 days suggested by Johnson (1996a), Wrigley and Fagg (1996) and Stewart and

Stewart (1999). However, variations are expected with germination being faster in warm, humid conditions and much slower in winter (Johnson, 1996a). It must also be noted that although the current experiment did take place over summer, the majority of experiments performed by other authors would have been done on mainland Australia where summer temperatures are higher, and were also probably using mainland species rather than *B. punicea.*

The maximum germination percentages reached by the mature *B. punicea* seeds in the current experiment were comparable to, and in fact higher than, the 90% mentioned by Johnson (1996a). Although there were no significant differences between the maximum germination percentages reached by seeds in light and dark treatments, differences did occur in the time to germination data. For both SC1 and SC3 seed lots, seeds reached maximum germination percentages faster in the dark than in the light, which again suggests light inhibition to germination or, alternatively, dark promotion of germination. Keith (1997) found that when seeds of *Epacris stuartii* were exposed to continual darkness, the rate of germination was also initially more rapid than those exposed to 14 hr light/10 hr darkness each day. Being able to germinate in the dark could be beneficial to *Blandfordia* seeds, which are quite small and may easily be covered by leaf litter or soil in their natural habitat. It has also been suggested that seeds that are germinating on the soil surface (i.e. seeds promoted to germinate by light) may be at greater risk of death due to desiccation than those germinating at depths where moisture is retained for longer (Keith, 1997), and that small-seeded species would be particularly susceptible to such losses (Harper, 1977).

This experiment also shows the importance of considering a number of factors when determining the best seed treatment. For the mature SC1 seed lot the highest germination percentage of 100% occurred in 4 treatments. Therefore, on germination percentage alone all of these treatments could be considered as the best. However, in a commercial situation where time is of high importance, the best treatment was 50% smoke dark. The seeds in this treatment only took 58 days to reach 100% germination. Control light seeds took 73 days, while 10% smoke light and 10% smoke dark both took 80 days to reach 100% germination. Therefore, although no

pre-treatment is necessary for *Blandfordia punicea* seeds, with 100% germination being reached in the control treatment, a 50% smoke dark treatment can promote faster germination. Smoke treatments have also been shown to promote earlier and more uniform germination in a range of other species (Dixon and Roche, 1995; Dixon *et al.*, 1995; Gilmour *et al.*, 2000; Morris, 2000). The germination rate, but not the final germination percentage, was affected by smoke in some of the species studied by Read and Bellairs (1999).

In conclusion, germination percentages of mature *B. punicea* seeds are not significantly increased or decreased by smoke. However, the naturally high germination levels that occur in this species are such that a seed pre-treatment is not required anyway. The post-fire behaviour of *Blandfordia* suggests that there would not be any adaptive significance to smoke promotion of germination. Following fire, *Blandfordia* can be categorised as a "re-sprouter" - it survives fire by forming new shoots from the buried rhizomatous corms (Johnson, *et al.*, 1994; Meney *et al.*, 1994), and flowers prolifically in the season following a fire (Johnson *et al.*, 1994). Therefore, it does not rely on seed stored in the soil to germinate following fire, instead, the seed enters an environment where the chance of a subsequent fire is extremely unlikely (Bell *et al.*, 1987), and as such there would be no adaptive significance to germination in response to smoke.

2.4.1.1.2 *B. punicea in vitro* light/ dark experiment

For the majority of species, seed germination is promoted by light (Mayer and Poljakoff-Mayber, 1975; Hartmann and Kester, 1983; Hilhorst and Karssen, 1989). However, germination may also be promoted by darkness, or seed may germinate indifferently to the presence or absence of light (Mayer and Poljakoff-Mayber, 1975; Vidaver, 1977). The effects of light may also vary according to temperature, especially in regard to diurnal fluctuations (Hartmann and Kester, 1983), the level of imbibition of the seed (Vidaver, 1977) and during storage - in some species a light requirement only exists immediately after harvesting, in others the effect persists for at least a year, while in others it only develops during storage (Mayer and Poljakoff-Mayber, 1975).

The germination response of many native Australian plants to light and darkness, often in combination with other treatments, have been studied (Bell, 1994; Bell *et al.*, 1995; Curtis, 1996; Keith, 1997; Morgan, 1998; Gilmour *et al.*, 2000). However, only limited work on the Liliaceae family has been undertaken (Morgan, 1998). In a study of comparative germination responses in *Themeda triandra* grasslands in southern Victoria, 6 Liliaceae species were included (Morgan, 1998). The germination percentage of one species, *Bulbine bulbosa*, was significantly inhibited by darkness, while the majority of species were unaffected by darkness (this was the case for *Arthropodium strictum*, *Burchardia umbellata* and *Dianella revoluta). Caesia calliantha* and *Wurmbea dioica* had such low germination percentages that no differences could be determined (Morgan, 1998).

With information lacking on many Liliaceae species' germination responses to light and darkness, and the differences between genera that occurred in Morgan's' (1998) study, it was thought relevant to determine whether any differences occurred between germination percentages of *B. punicea* when grown in the light or dark.

This experiment used the same South Cape seed lots used in the previous experiment, but this time they were surface sterilised and placed into *in vitro* conditions, where two different media types were used - an MS basal medium (Murashige and Skoog, 1962) and EC, a modified MS medium designed specifically for embryo culture (see Appendix 2.3 for media recipe). Seeds on both media types were placed in the light and the dark to germinate.

Again in this experiment there was no germination from any seeds in the SC2 seed lot, presumably due to the reasons discussed in the previous section (2.4.1.1.1).

Under *in vitro* conditions the SC1 control seeds performed similarly to the previous experiment where they were grown in petri dishes, with very high germination percentages (100%) reached in both light and dark treatments. In the previous experiment, the control dark seeds reached a slightly lower maximum percentage of 95%. Therefore, in control treatments both *in vitro* and *ex vitro* mature *B. punicea* seed will germinate equally as well in the light or the dark; as was the case for the majority of Liliaceae species in a previous study (Morgan, 1998).

The immature SC3 seed lot germinated better when grown *in vitro* than *ex vitro*, with the exception of the EC dark treatment, in which only 50% of seeds germinated. This was, however, slightly higher than the 40% that germinated *ex vitro* in the control light treatment. Interestingly, the trend towards better germination in the dark for immature *B. punicea* seeds in the control and 10% smoke solution treatments seen in the previous experiment, was reversed under *in vitro* conditions. Although there was no significant difference between the control (MS) seeds in the light and dark, when grown on the EC media results were significantly better in the light than the dark. A similar result also occurred in the SC1 seed lot when grown on EC media (but differences were not significant). Perhaps the dark response is linked to temperature. Light responses have been suggested as varying with temperature (Hartmann and Kester, 1983). In the *in vitro* experiment, temperatures would have been more constant and probably warmer than those experienced *ex vitro*. Perhaps immature *B. punicea* seeds respond better to light conditions at higher temperatures. Another point to consider is that the light intensities would have been higher in the incubation room than in the laboratory. It is possible that the seed was responding to these higher light intensities when immature. In the immature SC3 seed lot the seed coat is probably thinner than that of the more mature SC1 seed, and in some cases the seed was still green, which may allow penetration of more light, or different wavelengths of light, thus triggering the germination response. As this difference between germination percentages in the light and dark did not occur on the MS medium, but did occur in the more mature SC1 seed lot when grown on EC medium, there may also be an interaction between the constituents of this medium and light and/or temperature that is causing this effect.

The time it took for *B. punicea* seeds to begin germinating *in vitro* was only 1 day earlier than the previous *ex vitro* experiment. However, in the previous experiment the more mature SC1 seed began to germinate slightly earlier than the SC3 seeds, which was not the case in the current experiment. In fact, for both seed lots germination began at the same time (36 DAS) in all treatments except for EC dark, with the percentages being more than twice that of the SC1 seeds in the previous experiment, and actually being generally higher for the SC3 seed lot (varying

between 25-50%) compared to the 20-33% germination of the SC1 seed. Therefore, both seed lots began to germinate very rapidly under *in vitro* conditions and faster than those under *ex vitro* conditions in the previous experiment. This is expected as when seeds are placed *in vitro* all of the requirements for germination are present. They have a high amount of moisture available, the temperature is warm and conditions are humid; factors which have been previously noted as promoting faster germination in *Blandfordia* (Johnson, 1996a). The media also provides nutrients, which may aid in germination.

It is interesting to note that for the SC1 seeds, although the final germination percentages in the control light and control dark treatments were the same (100%), the control dark seeds took only 41 days to reach this maximum, while in the light, germination was more gradual, taking twice as long to reach maximum germination (80 days). Although the SC1 seeds on the EC medium reached a higher percentage germination in the light than the dark, those in the dark again reached maximum germination faster than those in the light (41 days c/f. 86 days). Rapid germination in the dark was also noted in the previous smoke solution experiment, and by Keith (1997) for *Epacris stuartii*. Perhaps this rapid germination in the dark has an adaptive role under natural conditions. If seed becomes buried it may germinate more rapidly to ensure that the developing seedling can reach the light it requires for further growth. If it didn't germinate rapidly it may become buried further in the soil meaning that when germination did take place the seedling may not be able to reach the soil surface before the energy reserves within the seed were used up. *Blandfordia* seeds are quite small and thus would not have the large energy reserves possessed by larger seeds.

The time range for seeds to reach maximum germination was smaller for the SC1 seeds than the SC3 seeds, which is expected as the more mature seeds are at the correct stage of development for germination to occur, and therefore would be expected to germinate in a more timely manner than the immature seeds, which would normally require further development before they are at the correct stage for germination to occur.

MS was a (significantly) more successful media type in terms of percentage germination for the mature SC1 seeds, while the EC media was (significantly) more successful for the immature SC3 seeds (but only in the light). This was to be expected as the EC media, being designed for the culture of embryos, was probably more suited to the immature seeds, with more sugar and a small amount of the auxin NAA present; while the constituents of the MS medium were more suited to the mature SC1 seeds, as this media was designed for growing mature plant tissues. However, the combination of the EC medium and the dark for the immature SC3 seeds actually appeared to inhibit germination, as results in both the light and the dark on MS medium were better than those grown on this treatment.

Similar to the smoke solution experiment, this experiment also supports the fact that *Blandfordia punicea* seeds do not require any seed pre-treatment (Johnson, 1996a), and final germination percentages showed that seeds were indifferent to light or dark conditions, which was the same as other members of the Liliaceae family including *Arthropodium strictum*, *Burchardia umbellata* and *Dianella revoluta* (Morgan, 1998). However, germination of mature seed can occur more rapidly if seed are grown on an MS medium in the dark. Although not available to all propagators, the use of *in vitro* techniques can ensure that germination is faster than by conventional methods, and even immature seed can reach 100% germination when grown in the light on a medium specifically designed for the culture of immature seed or isolated embryos.

2.4.1.1.3 *B. punicea in vitro* media type and kinetin experiment

When germinating seeds or growing plant parts *in vitro*, using the most appropriate media is crucial to success. This was highlighted in the previous experiment where MS medium was more suited to the mature *B. punicea* seeds and produced a higher germination percentage than the seeds grown on the EC medium.

This experiment was aimed at determining the best type of medium to germinate mature *B. punicea* seeds *in vitro*. The MS (control) and EC media used in the previous experiment were again used and compared with VFT medium - a half-strength MS medium supplemented with a 10μM concentration of the cytokinin 2iP.

It was thought that a half-strength medium may be more suitable for *B. punicea*, as it grows in infertile soils (Kirkpatrick, 1997). Another cytokinin, kinetin, which has been suggested as being able to overcome chemical inhibitors in seeds and after-ripening periods (see section 2.1.1.2.3), was also added to MS media at a range of concentrations to determine its effects on germination of *B. punicea* seeds.

When comparing the three main media types, seed germination reached very high levels on all types, with VFT being the best (100%). Perhaps this was due to the cytokinin in the media and/or the lower concentration of nutrients. The germination percentage of seed on the EC medium was lower than the control (MS) seeds (but not significantly), which was also the case in the previous experiment using a different seed lot. The results were comparable but slightly higher in this experiment (88% of seed germinated in this treatment in this experiment c/f. 80% in the previous experiment). Again, the reason for this is likely to be that the EC medium is tailored more to the needs of immature seeds and/or embryos and therefore does not promote germination of mature seeds as well as the MS medium does. The EC medium also contains a small amount of NAA, which is an auxin. Auxins have been known to inhibit germination at relatively low concentrations (Khan, 1977), which may explain why the germination percentage was reduced on this medium.

Three of the four treatments that reached 100% germination were supplemented with kinetin, which does suggest that this PGR may promote germination of *B. punicea.* The other treatment that reached 100%, VFT, was also supplemented with a cytokinin, 2iP, which also appears to produce a similar effect. However, it must be noted that the germination percentage in the four highest treatments was only slightly, and not significantly, different to the control treatment, again suggesting that a seed pre-treatment is not really necessary for *B. punicea* seeds, in accordance with the results of Johnson (1996a).

There were no general trends of germination percentage increasing with increasing kinetin concentration in the media. Instead, the lowest (0.5μM) and the two highest (32μM and 128μM) concentrations all produced 100% germination, while the 2μM and 8μM concentrations produced results lower than the control, and indeed lower than (or equal to in the case of 2μM kinetin) the EC media. Perhaps the seed only

respond to very low or very high concentrations of kinetin in the media, but this is unlikely. It is possible, but also unlikely, that the seed selected for these two treatments was somehow inferior to the seed used in the other treatments. As seed was randomly selected, it would indeed be unlikely that any seed that was inferior would all be allocated to the same treatment.

In this experiment seed took longer to begin germinating than in the previous two experiments (15 and 16 days longer than the previous experiments, respectively). This was most likely due to variation between the seed lots rather than climatic conditions, as although this experiment began almost two months later than the previous two experiments, the conditions within the incubation room should still have been constant, and therefore, the same as the previous *in vitro* experiment. It was interesting that the three treatments that produced the lowest germination percentages also took longer to begin germinating, with the MS + 8μM kinetin treatment (which had the lowest percentage germination) taking three weeks longer to start germinating than the treatments that were the first to begin germinating. Perhaps there was some kind of problem with the seeds in this treatment in particular, which slowed their germination and reduced the percentage of seeds that did germinate.

In this experiment the range of time taken to reach maximum germination was much larger than in the previous experiments. The fastest treatment in this experiment took 88 days, which was more than twice as long as the fastest treatments in the previous *in vitro* and *ex vitro* experiments, which both took 41 days. This is most likely due to the fact that the seeds which reached maximum germination fastest in the two previous experiments were germinated in the dark, while in this experiment all treatments were grown in the light. It may also be due to seed lot differences, or due to the seed degrading before it was used. This seed lot was kept for two months longer than the previous seed lot prior to being sown.

A number of seeds died after germinating in 6 of the 8 treatments, and it is not really known why. It does not appear to be linked to the concentration of cytokinin in the media, as the seeds grown on the highest concentrations only had a small proportion of seeds germinate and then die. It would be expected that the higher concentrations

might be too strong for the developing seedlings, but this was not the case. As the treatment with the lowest percentage germination also had a relatively high proportion of seedling death, it may have been due to inferior quality seed within this treatment.

There was no death of seedlings on the VFT and MS + 2μM kinetin treatments, and again, it is not really known why these seedlings had a better survival rate than the others. For the VFT media it could be suggested that the half-strength nutrient concentration of the media was better suited to the developing seedlings than the full strength concentrations in all other treatments. As noted previously, *B. punicea* naturally grows in nutrient poor soils (Kirkpatrick, 1997) so may be better suited to growth on the half-strength VFT media. However, this does not explain why all seedlings on MS + 2μM kinetin survived.

When all aspects of this experiment are considered, the best media treatment was probably MS + 128μM kinetin - 100% of seeds germinated, it had the most rapid germination rate of the treatments that reached this level of germination (taking 132 days) and, although some of the developing seedlings died, the proportion of dead seedlings was only very small.

In conclusion, the addition of kinetin to the media did not significantly increase the germination percentage of *B. punicea* seeds. However, as the seeds do not appear to have any dormancy issues this was to be expected.

2.4.1.2 *Dianella tasmanica*

2.4.1.2.1 *D. tasmanica* oven experiment

In previous experiments germination of *D. tasmanica* was found to be naturally low and sporadic (Sward, 1995), prompting suggestions that dormancy mechanisms, most probably mechanical due to the hard seed coat, or chemical caused by inhibitors in the fleshy fruit, were affecting germination. In order to overcome mechanical dormancy mechanisms, the current experiment using dry heat treatments was designed. Dry heat treatments have been successful in breaking dormancy in many native Australian species, particularly woody and herbaceous species with hard

seedcoats whose germination is promoted by heat from fire (Hodgkinson and Oxley, 1990).

For *D. tasmanica* a dry heat treatment did not increase germination percentages above those of the control seeds. In fact, treatment at 80°C and 100°C significantly decreased germination, with no germination occurring in these treatments. This suggests that these high temperatures actually killed the seeds. Experiments on a wide range of taxa indicate that maximum germination occurs after heat shock at 90°C for 10 min (Auld and O'Connell, 1991). However, other species, as well as *D. tasmanica* have been killed, or had their germination significantly reduced, by temperatures around 80°C. For example, *Patersonia sericea* (Iridaceae) had germination significantly decreased by heat at 80 - 85°C for 2 min, while *Xanthorrhoea gracilis* and *X. preisii* showed seed mortality following the same treatment (Bell *et al.*, 1987). Even hard-seeded *Acacia* and *Dodonaea* species had their seeds killed by slow burning litter fires when the temperature exceeded 80°C (Hodgkinson and Oxley, 1990). Germination of *Pimelea spicata* seeds was also inhibited by heat treatment at 90°C for 10 min (Willis *et al.*, 2003); while the seed of two large-seeded legume species from north eastern Australia, *Crotalaria pallida* and *Galactia tenuiflora*, was killed by a 5 min exposure to 100°C (Williams *et al.*, 2003).

Treatment at the lower temperature of 50°C did not kill *D. tasmanica* seeds, but the percentage of seeds that germinated in this treatment was exactly the same as the control seeds at the time the experiment was concluded. Therefore, this temperature was probably not high enough to crack the seed coat, which may have increased germination. A temperature between 50°C and 80°C or an increase in the exposure time may have been more successful. However, a heat treatment at 60°C for 1 hr did not increase germination of another Liliaceae species, *Thysanotus multiflorus*; and *Sowerbaea laxiflora* required a combinatorial treatment of smoke with heat to significantly increase germination levels. Heat alone (for extended periods) did not increase germination above that of the control (Tieu *et al.*, 2001). Also, species that are stimulated to flower following fire, like *Xanthorrhoea gracilis*, *X. preisii* (Gill, 1981a) and *Blandfordia* (Johnson *et al.*, 1994) (and perhaps *Dianella* and other

Liliaceae species) disseminate their seed in the season following fire. Therefore the seed enters an environment where the chance of a subsequent fire is extremely unlikely, and there is very little adaptive significance to being able to tolerate high temperatures (Bell *et al.*, 1987; Bell and Williams, 1998). This perhaps explains why the seeds were killed at temperatures greater than 80°C.

Although there was no difference between total germination percentages in the control and oven 50°C treatments, the seeds from the oven 50°C treatment began to germinate 35 days before the control seeds (79 DAS). Perhaps the hotter temperature stimulated some of the seeds to germinate faster than they would normally have. Even at 166 DAS, twice as many seeds had germinated from the oven 50°C treatment than the control treatment, and it was not until 206 DAS that the control seeds had reached the same number of germinated seeds as the oven 50°C treatment. Even though the number of seeds that had germinated was small, this faster germination, which occurred in the oven 50°C treatment, could be important in a commercial situation, where time is crucial.

The control seeds did not begin to germinate until 114 DAS, which was slightly slower than a previous experiment where untreated seed began to germinate 88 DAS (Sward, 1995). This may have been due to seasonal differences, such as temperature, or seed lot variability. In both cases germination was slow and sporadic. In the previous experiment days to germination varied from 88 to 188 days (and germination was still not complete) (Sward, 1995), while in the current experiment, control seeds began germinating at 114 days and were still continuing to germinate 534 DAS. Even though the Oven 50°C seeds began germinating faster than the control seeds, these seeds were also still germinating 534 DAS. Therefore, in general the oven 50°C treatment decreased the time to germination of *D. tasmanica* seeds early in the experiment, but did not change the sporadic nature of germination. It is interesting to note that the approximate time for total germination of *D. tasmanica* has been stated as only taking 83 days (Greening Australia, 1996b), which is very different to the results in this experiment. However, these results were obtained from experiments using seed from mainland plants of *D. tasmanica*, which may respond differently to seed from Tasmanian plants, and were carried out in controlled

temperature glasshouses, not under the natural diurnal temperature variations that the seeds were subjected to in the current experiment.

Only 38% of *D. tasmanica* seeds germinated in both the control and oven 50°C treatment. A similar low level of germination also occurred when untreated seeds were germinated previously (Sward, 1995). Only 20 to 30% of seeds germinated in seed lots from different locations, which suggested that this was a widespread phenomenon and not due to viability problems with seed from some locations. Seeds were also dissected to determine whether any embryos were present, and in 83% of these seeds, healthy embryos were found, thus determining that the majority of seed were viable (Sward, 1995). Victorian data suggests that germination of *D. tasmanica* seed should be much higher. Greening Australia (1996b) state that seed germinates well without fermentation, and that the typical number of germinants per gram is 127. Calculations based on the weight of *D. tasmanica* seeds from Tasmania show that this figure equates to approximately 78% of seeds germinating, which was much higher than the results from the current experiment. The reasons for this difference are probably due to seed lot variability between the mainland and Tasmanian seeds, and also due to the fact that the mainland experiments were performed under controlled environmental conditions, with constant and probably warmer, temperatures. Another Liliaceae species, *Burchardia umbellata*, has also shown differing germination levels in different regions of Australia. Western Australian populations of this species were found to be difficult to germinate, but could be promoted by smoke (Dixon *et al.*, 1995); while southern Victorian populations were readily germinable in both light and dark conditions (Morgan, 1998). However, it should be noted that this species has been stated as having a 2 - 3 month after-ripening period (Ralph, 1994; Greening Australia, 1996b), and in Morgan's' (1998) experiment all seed was stored at ambient temperature for 10 - 12 months prior to use. This storage period may be the reason that better results were achieved in this experiment. The smoke treatment may have overcome the after-ripening period in the experiment by Dixon *et al.* (1995).

The low natural rate of germination which occurred in this and previous experiments with *D. tasmanica* also appears to be a common feature in other monocot genera,

including *Anigozanthos* and *Macropidia* (Dixon and Hopper, 1979; Watkins and Shepherd, 1983; Goodwin, 1993; Sukhvibal and Considine, 1994; Worrall, 1996a) and species within the Liliaceae family (Morgan and Lunt, 1994) including *Thysanotus multiflorus* (Tan and Broadhurst, 1993), *Burchardia umbellata* (Dixon and Roche, 1995), *Caesia calliantha*, *Dianella revoluta* and *Wurmbea dioica* (Morgan, 1998); and *Patersonia* from the Iridaceae family (Dixon and Roche, 1995). Studies in the jarrah forest ecosystem of WA have shown that species with low levels of germination tended to be the long-lived species which survived fire by resprouting from underground bulbs, corms or rhizomes, and as such, where reproductive output may not be of major adaptive significance (Bell *et al.*, 1987), and this reasoning would also apply to *Dianella* and many of the other native Liliaceae and Iridaceae species.

The sporadic nature of germination also occurs in many other native Australian genera from a variety of families, such as: the "Flannel Flower", *Actinotus* (Offord and Tyler, 1996; Stewart and Stewart, 1999), from the Apiaceae family; *Persoonia*, family Proteaceae (Blombery and Maloney, 1994; Ketelhohn *et al.*, 1996); *Hardenbergia*, *Kennedia* (Stewart and Stewart, 1999), and *Swainsona*, the "Sturts' Desert Pea", (Williams, 1996a) from the Fabaceae family; *Boronia*, family Rutaceae (Plummer, 1996; Stewart and Stewart, 1999), *Ptilotus* (Williams, 1996b; Stewart, 1999b), family Amaranthaceae; and *Gahnia*, family Cyperaceae (Stewart, 1999b). For this phenomenon to be so widespread there must be an adaptive advantage. The sporadic pattern of germination is probably successful because it means that not all seedlings are growing at the same time, thus ensuring that all of the seedlings will not be destroyed by, for example, an extreme climatic event such as an out of season frost. It has also been suggested that species with slow and low germination such as *Bulbine bulbosa*, *Caesia calliantha*, *Dianella revoluta* and *Wurmbea dioica* (and perhaps other Liliaceae species) may be more likely to form persistent seed banks (Morgan, 1998). Although field evidence to support this proposition is largely lacking, *Caesia calliantha* seedlings have emerged from soil seed bank samples taken from long unburnt grasslands (Lunt, 1990; Morgan, unpublished data) and 46% of *Bulbine bulbosa* seeds reportedly survived for 1 year in a seed burial experiment

(Lunt, 1995). This proposition appears to make sense for species such as *Dianella tasmanica*, where seeds continued to germinate more than 500 DAS. With such a sporadic germination pattern the seed must be being stored somewhere, and if not within the soil, it would probably be consumed by predators. In contrast, seeds with high germinability or low innate dormancy (for example, *Blandfordia punicea)* have a germination biology that is not conducive to the formation of a large, persistent soil seed bank (McIntyre, 1990; Morgan and Lunt, 1994). Species that germinate almost completely (> 80%) in a short time period are most unlikely to persist in the soil (Morgan, 1998).

At present, the slow and sporadic pattern of germination seen in *D. tasmanica*, coupled with the very low germination percentage, would be totally unacceptable as a commercial method of propagation. As the oven treatment did not increase germination percentages, or decrease the sporadic nature of germination, other seed treatments were also investigated.

2.4.1.2.2 *D. tasmanica* fermentation experiment

D. tasmanica seeds are encased in a fleshy berry, which is possibly attractive to birds or animals (although there is no direct evidence of this) which eat the fruit and disperse the seeds. It is possible that this berry may contain chemical inhibitors (Robin, 1991; Blombery and Maloney, 1994) which prevent the seeds from germinating, and these inhibitors are removed by strong acids when the fruit passes through the animals' gut.

Fermenting fruit, so that the flesh rots away, while leaving the seeds intact, has been suggested as a way of removing these chemical inhibitors (Ralph, 1994) and has been successful for cycads and palms (Blombery and Maloney, 1994). In the current experiment, fermentation of seeds followed by a quick rinse in fresh tap water did increase the percentage of seeds that germinated, but the difference between the control and fermentation treatments was not significant. Only 38% of the control seeds germinated, which was exactly the same as both the untreated control and oven 50°C treatments in the previous experiment. This shows that approximately 38% is the natural level of germination of *D. tasmanica* seeds, at least in this seed lot.

The control seeds in this experiment began to germinate 125 DAS, which was only slightly longer than the control seeds in the previous experiment (which took 114 days). As mentioned in the previous section this is much longer than the 83 days to total germination cited by Greening Australia (1996b), and is probably due to the reasons mentioned in the previous section (2.4.1.2.1).

Unlike the oven 50°C treatment, which induced an earlier germination than the control seeds, the fermentation treatment actually caused seed to germinate much later than the control seed, taking more than twice as many DAS to begin to germinate (294 DAS c/f. 125 DAS for the control seed). It is not known why this would occur, but it must be noted that at the time the fermented seed began to germinate, a total of four seeds had germinated in this treatment, compared to six from the control. So there was not really a lot of difference between the number of seeds that had germinated at this time, and by the next recording date the number of germinated fermented seeds had actually surpassed the number of germinated control seeds.

In both treatments, the rate of germination was initially slow, with only a small number of seeds germinating until after about 351 DAS, when a rapid increase occurred within a 30 day period. This rapid rate of germination occurred in both treatments, but was more pronounced in the fermentation treatment. Perhaps it was at this point that germination inhibitors had been leached from the seeds by watering them, and as the seeds from the fermentation treatment had also been subjected to fermentation the level of inhibiting chemicals was already reduced, thus explaining the more rapid increase in germination seen in these seeds, compared to the control. Another reason may have been a change in climatic conditions, such as an increase in temperature, which may have stimulated the seeds to germinate. However, as the increase occurred in autumn (early April to early May) it would be unlikely that temperatures would be increasing. They would be more likely to be decreasing, which could also stimulate the seeds to germinate (depending on their optimum germination temperature).

As in the previous experiment, seeds were still germinating more than 500 DAS in both treatments, which shows that total germination takes much longer than

previously noted by Greening Australia (1996b). In both experiments, at the final recording dates, the germination had reached a plateau, so it was unlikely that seed would have continued to germinate much longer. However, 500 days to reach total germination is far too long to be a commercially viable method of propagation.

Although the difference between the control and fermented seed treatments was not significant, the fermentation treatment did induce a higher total germination percentage than the control, and is therefore quite promising and should be pursued. Perhaps if the fermentation process was followed by a period of leaching, germination inhibitors may have been removed more thoroughly, thus allowing seed to begin germinating earlier and to reach a higher level of germination.

2.4.1.2.3 *D. tasmanica* smoke experiment

Promotion of germination by smoke and smoke extracts has been a widely studied phenomenon throughout fire-prone regions of the world (see sections 2.1.1.3.3 and 2.4.1.1.1). Preliminary research showed that seeds of *D. tasmanica* were responsive to smoke (Sward, 1995) and it was thought prudent to further investigate smoke treatments as a means of increasing germination percentages of this difficult to germinate species.

Smoke treatments (both direct and in liquid extract form) increased the percentage germination of *D. tasmanica* seeds compared to the control (with the exception of 100% smoke, which significantly inhibited germination). Smoke extracts were also found to increase the percentage germination of *D. tasmanica* seeds in a preliminary experiment (Sward, 1995); and the closely related *D. revoluta* has also been reported as showing a positive germination response to smoke (Greening Australia, 1996a; Stewart and Stewart, 1999). Dixon and Roche (1995) and Dixon *et al.* (1995) also found that other monocotyledon genera that normally have low germination percentages were positively stimulated by smoke. These genera included *Anigozanthos* and *Conostylis* (Haemodoraceae), *Thysanotus* and *Burchardia* (Liliaceae) and *Patersonia* (Iridaceae). A number of other Liliaceae species have also been listed as showing positive germination responses to smoke and/or smoke

extracts, eg. *Arthropodium* sp., *Burchardia umbellata, Caesia calliantha* and *Sowerbaea laxiflora* (Greening Australia, 1996a; Stewart and Stewart, 1999).

In the current experiment direct smoke was more effective in promoting germination of *D. tasmanica* seeds than smoke extracts. Aerosol (direct) smoke also had a greater stimulatory effect on germination of seeds from the soil seed bank in jarrah forests in WA (Roche *et al.*, 1997b). However, the difference between the percentage germination of seeds from the direct smoke and 50% smoke extract treatments was not significant. It is possible that a higher concentration of smoke extract (between 50% and 100%) may have elicited a response equal to, or perhaps greater than, that obtained in the direct smoke treatment.

In both the current and the preliminary (Sward, 1995) smoke extract experiments, the percentage germination of *D. tasmanica* seeds increased with increasing smoke extract concentration (with the exception of 100% smoke in the current experiment). In the preliminary experiment the highest concentration used (10% smoke) produced the best germination (50%); while in the current experiment the highest concentration used (that did not inhibit germination) (50% smoke) produced the best result (78.3%). In both experiments the 1% smoke solution produced a similar percentage germination to the control treatment. Therefore, this solution is probably too dilute to have any positive effect. The 10% smoke solution caused a similar percentage of *D. tasmanica* seeds to germinate in both experiments (50% in the preliminary experiment and 48.3% in the current experiment), which suggests that this level of germination could also be expected in further experiments. A slightly higher percentage of seeds germinated in the control and 1% smoke treatments in the current experiment than the preliminary one, but differences were only slight and can probably be attributed to seed lot variation. The percentage germination of control and 1% smoke seeds were both 20% in the preliminary experiment, while in the current experiment control seeds reached 35% germination and 1% smoke seeds reached 38.3%. As only a small number of seeds were used in the preliminary experiment, the results from the current experiment are more likely to be correct. Also, the percentage of control seeds that germinated in the current experiment

(35%) was similar to the 38% of control seeds that germinated in both the oven and fermentation experiments, which further suggests that they are correct.

As mentioned previously, the undiluted (100%) smoke extract inhibited germination of *D. tasmanica* seeds. This was also the case for *I. tasmanica* seed and the immature (SC3) *Blandfordia punicea* seed. Inhibition of germination by higher concentrations of smoked water has also been noted by other authors for a range of different species (Brown, 1993a; Baxter *et al.*, 1994; Brown *et al.*, 1994a; Dixon and Roche, 1995; Drewes *et al.*, 1995; Roche *et al.*, 1997a; Gilmour *et al.*, 2000).

Smoke treatments were also found to promote faster germination of *D. tasmanica* seeds (with the exception of 100% smoke). Direct smoked seeds began to germinate first, 65 DAS, which was also the case in a preliminary experiment (Sward, 1995). However, the direct smoked seeds in that experiment took slightly longer to begin germinating (102 DAS). Smoke extract treated seeds also began germinating prior to the control plants, and with increasing smoke extract concentration the number of days until seeds began to germinate decreased. Seeds from the 50% smoke extract treatment began to germinate within a week of the direct smoked seeds (72 DAS). Therefore, these treatments were similar in terms of time to germination. Smoke treated seeds of *Blandfordia punicea* (50% smoke in combination with a dark treatment) also germinated faster than the control seeds (see section 2.3.1.1.1). In the preliminary smoke extract experiment the majority of *D. tasmanica* seeds in the 5% smoke and 10% smoke treatments also germinated sooner than the control seeds. Dixon and Roche (1995) and Dixon *et al.* (1995) also found that first germination events occurred noticeably earlier in smoke treatments than in controls in the following species: *Grevillea wilsonii*, *Andersonia lehmanniana*, *Hypocalymma angustifolia*, *Petrophile drummondii* and *Stackhousia pubescens*. Smoke treated *Grevillea linearifolia*, *G. diffusa*, *G. mucronulata* and *G. speciosa* seeds also germinated earlier than control seeds (Morris, 2000).

The time taken to reach maximum germination was also faster for smoke treated *D. tasmanica* seeds than control seeds (with the exception of 100% smoke). The sporadic natural germination pattern, in which seeds were still germinating more than 500 DAS (control) was reduced, with maximum germination occurring as early as

191 DAS (direct smoke). Smoke treatments have also been shown to promote earlier and more uniform germination in a range of other species (Dixon and Roche, 1995; Dixon *et al.*, 1995; Gilmour *et al.*, 2000).

The fact that the undiluted (100%) smoke extract both reduced the percentage of seeds germinating and also increased the time taken to begin germinating suggests that this concentration is too high and is adversely affecting the seeds. As mentioned previously, many other species have also been adversely affected by high concentrations of smoked water.

In conclusion, germination percentages of *D. tasmanica* were generally increased by smoke treatments, with direct smoke being more effective than smoke extract treatments. The time taken to begin germinating and to reach maximum germination was also reduced by smoke treatments, an important factor in a commercial situation where time can be crucial.

2.4.1.2.4 *D. tasmanica* partial removal of testa experiment

Due to the low natural germination percentage of *D. tasmanica* seeds it has been suggested that dormancy mechanisms are affecting germination in this species (Sward, 1995). These mechanisms are thought to be either mechanical, due to the hard testa, or chemical, caused by inhibitors in the fleshy fruit. The results from the fermentation experiment (in which percentage germination was not significantly improved by a fermentation treatment) suggest that the dormancy is not of a chemical nature. Therefore, this experiment was designed to investigate the possibility of mechanical dormancy affecting germination in this species.

Partial removal of the testa (and subsequent growth *in vitro*) significantly increased the germination percentage of *D. tasmanica* compared to the control. This treatment elicited the highest germination percentage (90%) for this species in both the current and previous (Sward, 1995) studies. As none of the control seeds germinated *in vitro*, this high germination response can be solely attributed to the partial removal of the testa, which supports the suggestion that the dormancy mechanism preventing germination of *D. tasmanica* seeds is a mechanical one caused by the hard testa. It is

also likely that the hard testa acts as a permeability barrier to germination, preventing the movement of water and/or oxygen into the seed.

Partial or entire removal of the testa has also significantly increased percentage germination of three species from the native Australian Dilleniaceae genus *Hibbertia*, which also has a hard testa. *H. hypericoides* (Schatral, 1996), *H. commutata* and *H. amplexicaulis* (Allan *et al.*, 2004) all had very low natural germination percentages which were significantly increased by removal of the testa. This method has also been suggested for the native Iridaceae genus, *Patersonia* (Ralph, 1994).

As mentioned previously, none of the control seeds germinated *in vitro*, which was unusual as germination percentages of up to 38% were noted for control seeds in previous experiments. However, in a preliminary *in vitro* experiment there was also a lack of germination after 112 days in culture (Sward, 1995). As seeds in the current experiment were only observed for 90 days, it is possible that germination may have occurred later. In the oven and fermentation experiments control seeds did not start to germinate until 114 DAS and 125 DAS respectively. Therefore, had observations taken place until approximately 130 DAS germination may have occurred.

As well as achieving the highest percentage germination observed for *D. tasmanica* in the current study, the germination rate was also significantly faster when the testa was partially removed. Seeds began to germinate after only 12 days *in vitro*, compared with 79 DAS for oven treated (50°C) seeds, which was the fastest of all other treatments investigated for this species. The maximum germination percentage (90%) for the partial removal of testa treatment was reached after only 32 days; while for the oven and fermentation treatments (as well as the control seeds from both of these experiments) seeds were still germinating more than 500 DAS.

It should also be noted that an even higher germination percentage than the already high 90% may have been achieved using the partial removal of testa treatment. As this treatment was quite difficult to apply to seeds without damaging the tissues inside, it is possible that the seeds that did not germinate may have had their embryos damaged.

In conclusion, by removing part of the testa of *D. tasmanica* seeds the germination percentage can be significantly increased, which supports the theory that the dormancy imposed on these seeds is a mechanical one, caused by the hard testa. This finding has huge implications for the horticulture industry, as it means that this species can now be germinated quickly and to much higher percentages than previously obtained. Importantly, new material from breeding programs will reach the marketplace in a timelier manner.

2.4.1.3 *Milligania densiflora*

2.4.1.3.1 Initial *M. densiflora* seed germination experiment

The literature describing seed germination of *Milligania* is scarce, perhaps due to the genus' endemicity to Tasmania. An initial seed germination experiment was performed to determine the natural germination levels of *M. densiflora*, and to show whether storage of seed had any effect on germination. Seed from two locations was used, with one lot being collected from plants growing at the MBG, vacuum sealed and then stored at 4°C, and the other lot being freshly collected from Mt Read, Tasmania. Seed from both locations was then placed in the laboratory or in an incubation room to germinate.

Seeds from the MBG failed to germinate, regardless of their location, while Mt Read seeds germinated in both the laboratory and the incubation room. This suggests that either fresh seed germinates better than stored seed, which is apparently the case with other Liliaceae genera, including *Dianella* (Elliot and Jones, 1984; Ralph, 1994) and *Blandfordia* (Collier, 1991; Stewart and Stewart, 1999; Stewart, 2002), or, that there was some kind of viability problem with the MBG seeds (which could be associated with storage - older seed may be less viable than fresh seed).

The Mt Read seeds reached a higher germination percentage in the incubation room than the laboratory (75% c/f. 48% respectively). This is probably due to the constant and higher temperature that seeds would have been subjected to in the incubation room, and/or the more intense lighting in the incubation room, compared to the natural light in the laboratory. *M. densiflora* is an alpine species and the seed have

probably adapted to germinating in the high intensity light and warmer temperatures experienced by alpine regions in the summer (Collier, 1991), when it would be naturally germinating.

Compared to the other Liliaceae species studied in this project, *M. densiflora* seeds began to germinate very quickly, after only 17 days, which was even faster than the *Blandfordia punicea* seeds (which began to germinate between 36 and 52 DAS). Due to the physical similarities of the seeds, it may have been expected that *M. densiflora* seeds would perform similarly to *D. tasmanica* seeds, but this was not the case. It appears that the dormancy mechanisms inhibiting *D. tasmanica* seeds from germinating are not present in *M. densiflora* seeds.

The rate of germination was initially more rapid in the seeds growing in the incubation room, which is probably due to more favourable germination conditions for this species, as discussed previously. Maximum germination appeared to have been reached by the seeds growing in the laboratory at approximately 150 DAS. However, the pattern of the graph suggests that the incubation room seeds were continuing to germinate at this time, and may have gone on to reach a higher percentage than the already high 75% (perhaps even reaching 100% germination), had the experiment continued. Unfortunately, a fungal contaminant overtook the seeds in the incubation room and the experiment had to be abandoned at this point.

The time to maximum germination of *M. densiflora* is quite quick (approximately 150 DAS) compared to the slow and sporadic germination of *D. tasmanica*, which was still occurring more than 500 DAS. However, it was slower than *B. punicea*, which is probably expected as *B. punicea* has a much softer seed coat than *M. densiflora* and probably imbibes water faster and/or does not last very long after removal from the seed capsule due to factors such as desiccation, so must germinate quickly.

M. densiflora appears to have a fairly high natural germination level, especially when subjected to warmer temperatures (approximately 25°C) and a high light intensity, which would be similar conditions to its natural alpine habitat during summer, when seed germination would be occurring. Seed germination appears to be

a suitable method of propagation for this species in a commercial sense, as seeds reached a fairly high germination percentage and germinated quite quickly. There were more germination aspects of *M. densiflora* that were still unknown. Therefore, further germination experiments were investigated.

2.4.1.3.2 Seed germination of *M. densiflora in vitro*

To investigate seed germination behaviour of *M. densiflora* further and to provide material for *in vitro* propagation experiments, *M. densiflora* seeds from the same seed lots used in the previous experiment were disinfested and placed in *in vitro* conditions.

The results were confounded by contamination problems, particularly for Mt Read seeds, where 70% of seeds became contaminated within 17 days of placement on the media. As contamination occurred so quickly it suggested that the disinfestation procedure was not sufficient to remove contaminating microorganisms from the seed surface.

As in the previous experiment, *M. densiflora* seeds from MBG again failed to germinate. If seed would not germinate under *in vitro* conditions, where all requirements for germination are met, then it is likely that the seed is not viable, further suggesting that fresh seed must be used for optimal germination of *M. densiflora*. Indeed, the fresh seed from Mt Read did germinate, with the first seed germinating 17 DAS, exactly the same time as seeds began to germinate in petri dishes in the previous experiment. The time taken to reach maximum germination was also very similar to the previous experiment, which suggests that the time to total germination for *M. densiflora* is approximately 150 days.

The percentage of seeds that germinated from Mt Read appears to be quite low (22%). However, this result was confounded by the high level of contamination that occurred. When the contaminated seed is not included, the germination was actually very high (88%), and probably comparable to the germination percentage which may have been expected in the first experiment had the seeds not become contaminated.

Survival of the Mt Read seedlings was quite poor. Only 50% survived, with the other half dying soon after germination. This phenomenon also occurred in *B. punicea* seedlings *in vitro*, and can probably be explained with the same reasoning. Similar to *B. punicea*, *M. densiflora* naturally occurs on soils which are nutrient poor (Kirkpatrick, 1997). The concentration of nutrients in the MS medium is probably too strong and therefore toxic for these species which have adapted to growth in the presence of low nutrients. The seedlings that survived may have been stronger and therefore more able to cope with the high nutrient concentrations. Alternatively, the death of seedlings may have been due to a growth-inhibiting chemical being present within the seed. This was the case with *Thysanotus multiflorus* (Tan and Broadhurst, 1993). In germination experiments with this species, nicking was found to significantly increase the percentage of seeds germinating, but the majority of seedlings did not survive after germination. This problem was solved by agitating nicked seeds in a beaker of water for a day; thereby suggesting that the harmful substance was soluble and could easily be removed by leaching (Tan and Broadhurst, 1993).

This experiment showed that germination of *M. densiflora in vitro* was possible, providing that fresh seed was used. Germination *in vitro* took approximately the same time as *ex vitro*, and the percentage germination was very high when the confounding effects of contamination were removed.

2.4.1.3.3 Seed germination of *M. densiflora in vitro* when disinfested for different times and placed on two media types

The high contamination rates that occurred in the previous experiment, together with the poor survival of seedlings following germination, prompted a second *in vitro* experiment. In this case seeds were disinfested in 2% NaOCl for either 20 or 35 min (longer than the unsuccessful 15 min used in the previous experiment) and they were then placed on either a basal MS or a 1/2 strength MS medium. It was hoped that a 1/2 strength medium may increase the survival rate of seedlings.

2.4.1.3.3.1 Contamination

Contamination rates were reduced significantly (by approximately 50%) when seeds were disinfested in 2% NaOCl for 35 min instead of 20 min. Therefore this longer time period was necessary to remove contaminating microorganisms from the seed surfaces. However, it was not entirely successful, as some seeds still became contaminated, so this disinfestation period could be extended further. It must be noted that exposure to NaOCl for extended periods may be detrimental to the seeds, so further experimentation is required to determine the optimum disinfestation time. Rather than extending the exposure time, the NaOCl concentration could instead be increased to perhaps 4%, which may disinfest the seeds properly in a shorter time period.

2.4.1.3.3.2 Germination

Germination began 16 DAS, one day earlier than the two previous experiments. Therefore *M. densiflora* seeds generally begin germinating 16 - 17 DAS, in both *in vitro* and *ex vitro* conditions.

Germination percentages were quite high for all treatments, and even reached 90% in the 1/2 MS (35 min) treatment. Therefore, this combination of the longer disinfestation procedure and the 1/2 strength medium appears to be the most suitable for germination. When seeds were disinfested for only 20 min the percentage germination was similar, regardless of the media type the seeds were grown on. However, when disinfested for 35 min the percentage germination was higher than the 20 min treatment on both media types. Perhaps the exposure to 2% NaOCl for 35 min may chemically scarify the seed and consequently improve germination. Bleaching agents, including sodium hypochlorite, calcium hypochlorite and hydrogen peroxide have been suggested as chemical scarification treatments (Ralph, 1994) and it is possible that the sodium hypochlorite is acting in this way. Treatment with 0.7% NaOCl improved germination of *Anigozanthos manglesii* by eliminating the physico-chemical barrier to germination (Sukhvibul and Considine, 1994).

2.4.1.3.3.3 Seedling survival

In contrast to the previous experiment where seedling survival was poor, all of the seedlings in this experiment survived. If there was a growth-inhibiting substance present within the seeds, perhaps the longer exposure time to the NaOCl may have been enough to leach the inhibitor from the seeds. As seedling survival was the same on both media types it does not appear to have been due to nutrient or growth factor concentrations within the media, as previously thought.

In conclusion, this experiment showed that *M. densiflora* seeds should be disinfested for at least 35 min in 2% NaOCl, and probably longer, to reduce contamination levels. The 1/2 MS medium was the most suitable for germination, but only when in combination with the 35 min disinfestation period.

2.4.2 Iridaceae Seed Germination Experiments

2.4.2.1 *Diplarrena moraea*

2.4.2.1.1 *D. moraea* initial *in vitro* seed germination experiment

Diplarrena moraea seeds are flat, without hard external testa's and it is therefore unlikely that there are any dormancy mechanisms, especially physical, operating. It would be expected that the seed would germinate readily, but information on the topic is scarce with most authors only stating that propagation is by seed (Elliot and Jones, 1984; Collier, 1990; Ralph, 1994; Wrigley and Fagg, 1996). Therefore an initial experiment was performed to determine the natural germination levels for *D. moraea* seeds. It was done *in vitro* so that seedling material could be used for micropropagation studies.

The initial experiment was very poor in terms of germination. Only one seed germinated, but this can probably be attributed to the fact that the seed was not fresh. The seed that did germinate did so very quickly - only taking five days, which would probably be expected due to the physical characteristics of the seed (as described above). The unhealthiness of the developing seedling could be linked to the age of

the seed, to an incompatibility with nutrient concentrations or growth factors within the media, or perhaps due to a growth-inhibiting substance within the seed (see section 2.4.1.3.2).

Contamination levels were also a problem in this experiment, especially for the seeds that were disinfested in the weaker 1% NaOCl solution (only 17% of seeds were not contaminated). This weaker solution was tested due to the apparent fragility of the seeds. However, the stronger 2% NaOCl solution was more successful, with 67% of seeds being free of contamination, and it also did not appear to damage the seed (the one seed that germinated was disinfested with the 2% NaOCl solution). Although unsuccessful in terms of germination, the results from this experiment were very useful. They suggest that seed must be fresh to germinate, and for further *in vitro* work, seed should be disinfested with 2% NaOCl for at least 15 min. A longer exposure to NaOCl would probably be more successful in terms of removal of contaminating microorganisms. However, care must be taken not to damage the seeds.

2.4.2.1.2 *D. moraea* second *in vitro* seed germination experiment

Due to the problems encountered in the initial *D. moraea* experiment a second *in vitro* germination experiment was done. This time fresh seeds were used and the disinfestation period was increased to 20 min in 2% NaOCl. This slight increase in the time that seeds were exposed to the NaOCl solution was completely successful - 100% of the seeds were free of contamination for the duration of the experiment.

The percentage germination was very high (97%) showing that *D. moraea* seeds germinate very well without any seed pre-treatment. This was expected due to the physical characteristics of the seeds. The seeds began to germinate very quickly, taking only 10 days, which was quicker than any of the Liliaceae species studied. Again this should be expected as the seeds are very flat, without a hard testa, and would probably take less time to imbibe than the larger and thicker seeds of *D. tasmanica* and *M. densiflora* (in particular).

The germination rate of *D. moraea* was initially very rapid, with the majority of seeds (88%) germinating within 51 days. There were a few seeds that did not

germinate until nearly 200 days later. Perhaps these seeds were not as viable as the initial germinants, or were slightly immature when the experiment began, or, this species may also be displaying a strategy where not all seeds germinate at once, thus ensuring that all seedlings are not destroyed by an extreme climatic event or similar natural disaster. In any case the majority of seeds germinated very quickly. Considerably quicker than the Liliaceae species tested, except for some treatments of *B. punicea*, which were slightly quicker (41 days).

Therefore, a disinfestation period of 20 min in 2% NaOCl is successful in removing contaminants from *D. moraea* seeds, and the natural germination rate is very high showing that seeds don't require any pre-treatment. Propagation by seed would definitely be a viable option for commercial production of this species.

2.4.2.2 *Diplarrena latifolia*

2.4.2.2.1 *D.latifolia in vitro* media type and kinetin experiment

D. latifolia seeds are almost identical to *D. moraea* seeds and it was therefore expected that they would have similar germination behaviours. The same disinfestation protocol was followed, and although not 100% successful, the contamination levels were low, with no more than two seeds contaminated in each treatment (and generally lower). Therefore, this protocol can still be recommended for further *in vitro* seed germination experiments for this genus.

The first part of this experiment was aimed at determining which media type (MS or EC) was most suitable for *D. latifolia*. The effects of light and dark on germination were also incorporated into this experiment.

The first seed to germinate did so in 10 days, which was exactly the same as the *D. moraea* seeds in the previous experiment, suggesting that the two species do have similar germination patterns. All treatments in the light began to germinate 10 DAS but it appears that dark conditions slow germination, as these seeds did not begin to germinate until 25 DAS. Seeds also reached higher germination percentages in the light than the dark on both media types and, although differences were not

significant, dark conditions do appear to slightly inhibit germination in terms of both time to germination and percentage germination.

Seeds grown on MS media reached higher germination percentages than those grown on EC media (in both light and dark conditions), although only significant for MS light. This was expected as the seeds appeared to be fully mature and would therefore be better suited to the constituents of the MS media, rather than a medium designed for growth of immature embryos. Mature *B. punicea* seeds also germinated better on MS than EC media (See Section 2.3.1.1.2).

The second part of this experiment studied the effects of the PGR kinetin on the germination of *D. latifolia*. Kinetin has been suggested as being able to overcome chemical inhibitors in seeds and after-ripening (Ralph, 1994; Stewart, 1999b), and it was thought that it may increase germination percentages as it did in *B. punicea* seeds (see Section 2.3.1.1.3).

In general, the presence of high concentrations of kinetin in the media increased germination percentages of seeds compared to the control (but not significantly). MS + 8μM kinetin and MS + 128μM kinetin produced the best results (90% and 89% respectively). In the similar experiment with *B. punicea* seeds (see Section 2.3.1.1.3), MS + 128μM kinetin also produced the highest percentage germination (together with four other treatments). At the lowest kinetin concentrations (0.5μM and 2μM) germination percentages of *D. latifolia* were actually less than the control seeds. A similar result occurred in the *B. punicea* kinetin experiment, except in this case the seeds grown on the mid-range kinetin concentrations (2μM and 8μM) had lower germination percentages than those grown on the lowest and the two highest kinetin concentrations. Therefore, kinetin, especially at high concentrations, does appear to increase the number of seeds germinating compared to the control treatment, but further experimentation is required. Perhaps a higher kinetin concentration than the 128μM used in this experiment would further increase the number of seeds germinating.

A number of seeds died after germinating in all treatments, except for MS + 2μM kinetin. However, the proportion of dead seedlings was generally low and there were

no significant differences between treatments. This phenomenon also occurred in the *B. punicea* experiment (see Fig. 2.5) and interestingly, in that experiment, all of the seedlings on the MS + 2μM kinetin treatment also survived. As was the case with *B. punicea*, the proportion of dead *D. latifolia* seedlings also does not appear to be linked with kinetin concentration in the media, as the seeds grown on the highest concentrations only had a small proportion of seeds that germinated and then died. It would be expected that the higher kinetin concentrations might be too strong for the developing seedlings, but this was apparently not the case. As the treatment with the lowest percentage germination also had a relatively high proportion of seedling death, it may have been due to inferior seed quality in this treatment. Interestingly, the *B. punicea* treatment with the lowest germination percentage also had one of the highest proportions of dead seedlings. This may suggest that a chemical inhibitor is present within the seed that is toxic to the developing seedlings (see Section 2.4.1.3.2).

The fact that the MS light treatment had quite a high proportion of seedlings that germinated and died may point to the media itself as a reason for seedling death. All treatments used a basal MS medium with the addition of other chemicals. Perhaps the nutrient or growth factor concentrations within the media are too strong for the developing seedlings, and a half strength media, for example, may be more suitable. However, this does not explain why all of the seedlings grown on the MS + 2μM kinetin treatment survived.

As well as increasing the percentage germination of *D. latifolia* seeds, a high concentration of kinetin in the media generally sped up the germination process, with the treatments that had the highest germination percentages reaching maximum germination quite quickly compared to the control. Therefore, the effects of kinetin on germination of *D. latifolia* seeds appear to be promising and should be further investigated.

When all aspects of this experiment are considered, the best media treatment was probably MS + 128μM kinetin - 89% of seeds germinated, taking 111 days (quite early in the range of 94 to 158 DAS that treatments took to reach germination

capacity); and although some of the developing seedlings died, the proportion of these was only very small (12.5%).

The results of this experiment show that *D. latifolia* germinates quite well *in vitro* without any seed pre-treatment, but with the addition of kinetin, germination can be increased further and is more rapid. With or without a seed pre-treatment the percentage germination would be high enough to be viable as a commercial method of propagation.

2.4.2.3 *Isophysis tasmanica*

2.4.2.3.1 *I. tasmanica* light/dark experiment *in vitro*

The literature describing seed germination of *Isophysis* is scarce, and this is probably due to its endemicity to Tasmania and restricted distribution. Apart from a few reports stating that the genus can be propagated by seed (Ralph, 1994; Wrigley and Fagg, 1996) no further detailed information could be found. Therefore an initial experiment was done in order to determine the natural germination level of *I. tasmanica*. The experiment was done *in vitro* so that seedlings could be used for further *in vitro* propagation studies, and incorporated light and dark conditions to determine their effects on germination.

The disinfestation protocol was 100% successful which means that it can be used for further *in vitro* seed germination experiments.

Seeds began to germinate 48 DAS, which was much slower than *Diplarrena*, the other Iridaceae genus studied, which only took 10 days. However, this could be expected as the *Diplarrena* seeds are much flatter than the angular *Isophysis* seeds and probably imbibe faster. In comparison to the Liliaceae species studied, *I. tasmanica* was also slower than *M. densiflora* (which took approximately 16 - 17 days to start germinating). It was faster than the *D. tasmanica* seeds (control seeds took 114 and 125 days to start germinating) and similar to *B. punicea*, which took 36, 37 and 52 days in the three experiments performed. In terms of external seed characteristics the angular *Isophysis* seeds are also most similar to the brown, angular *Blandfordia* seeds.

Dark conditions appeared to promote germination in terms of percentage germination, the time taken to begin germinating and the time taken to reach maximum germination. Although the number of seeds used was only quite small, there was quite a large and significant difference between the number of seeds that germinated in the dark (100%) compared to the light (40%). Consequently, the promotion of germination by dark conditions should definitely be pursued. In addition, the seedlings that germinated in the light all looked unhealthy and appeared to be dying, while those that germinated in the dark (and were then transferred to light conditions approximately one week after germinating) were all still green and healthy. This further suggests that *I. tasmanica* seeds have adapted to germination in the dark. Under natural conditions, as the seeds are quite small, they would be easily buried by soil or leaf litter or perhaps fall into crevices between rocks. Therefore, the ability to germinate in the dark would be of adaptive significance. It is also possible that the emerging seedling is too weak to survive such a high light intensity (as in the incubation room); but once it is larger and would be emerging from the soil (such as the dark germinated seedlings that were then placed in the light approximately one week after germinating) it is able to cope with the high light intensity.

2.4.2.3.2 *I. tasmanica* smoke solution experiment

After the initial experiment suggested that germination was promoted by dark conditions *in vitro*, a second experiment was designed to test this theory *ex vitro* and also to investigate the effects of different concentrations of a smoke extract solution on germination of this species.

Seeds began to germinate 54 DAS in the majority of treatments. This was only slightly longer than the seeds took to begin germinating *in vitro* in the previous experiment. The *in vitro* seeds probably germinated faster as the microclimate *in vitro* satisfies all requirements for seed germination - there is abundant moisture, the temperature is warm and humidity is high.

As was the case *in vitro*, seeds also germinated more rapidly when grown in the dark *ex vitro* (with the exception of the 100% smoke dark treatment). This further suggests that *I. tasmanica* seeds germinate better in the dark than the light. Rapid

germination in the dark was also noted for *B. punicea* in the smoke solution and *in vitro* light and dark experiments. It has also been noted for species from other families, such as *Epacris stuartii* (Keith, 1997).

The percentage germination of seeds in all treatments was very high (> 90%), with the exception of the 100% smoke light and dark treatments. This suggests that the undiluted smoke solution was too strong for the seeds and actually inhibited their germination. This inhibition of germination has been noted in other species when strong concentrations of aqueous smoke are used (Brown, 1993a; Baxter *et al.*, 1994; Brown *et al.*, 1994a; Dixon and Roche, 1995; Dixon *et al.*, 1995; Drewes *et al.*, 1995; Roche *et al.*, 1997a; Gilmour *et al.*, 2000). The inhibition was more pronounced in dark conditions than light, which shows that at a strong smoke concentration, the promotive effects of dark on germination are actually reversed. This must be due to some interaction between these two factors. It must also be noted that fungal contamination may have confounded the germination results in the 100% smoke dark treatment, as half of them were mouldy 94 DAS. However, one mouldy seed did later germinate, so the presence of the mould did not prevent germination of this seed, but may have done for others.

There were no other significant differences between treatments. For both the control and 10% smoke treatments, germination was slightly higher in the dark than the light, but not significantly. In the 50% smoke treatments germination was exactly the same in the light and dark (100%). So the promotive effect of dark on percentage germination is probably limited to *in vitro* conditions. Perhaps, as alluded to in the previous section, the high light intensity *in vitro* may actually be inhibiting germination. In *ex vitro* conditions the light intensity is more natural and perhaps not strong enough to inhibit germination.

Although dark conditions don't appear to increase the germination percentage *ex vitro*, it must be noted that seeds began to germinate sooner in the dark, and reached maximum germination 79 days before the seeds in the light for all treatments (except for 100% smoke). Therefore dark conditions must still be recommended for germination of *I. tasmanica.*

This experiment showed that *I. tasmanica* seeds reach very high germination percentages (90 to 100%) without any seed pre-treatment. Therefore propagation by seed would be a commercially viable option for this species. Aqueous smoke solutions did not improve germination, and actually inhibited germination when undiluted. Dark conditions did not significantly increase total germination percentages but did speed up the germination process, with seeds starting to germinate earlier, and reaching maximum germination faster, than those in the light.

2.5 Summary and Future Research

Propagation by seed is possible for all of the species included in this study, with germination occurring in all experiments. The natural germination percentages varied between species, as did the time from sowing until germination began and the time taken to reach maximum germination. Propagation by seed would be a viable commercial option for all species, with no pre-treatments required for some species (eg. *Blandfordia punicea*), but definitely required for others (eg. *Dianella tasmanica*).

2.5.1 Liliaceae species

2.5.1.1 *Blandfordia punicea*

- Immature *B. punicea* seeds did germinate, but did not reach as high germination percentages as the mature seed.
- Mature seed also began to germinate sooner than the immature seed.
- The natural germination percentage of mature *B. punicea* seeds was extremely high in all experiments both *in vitro* and *ex vitro* (100% in two experiments and 93% in the third).
- Treatment with smoke extract solutions did not significantly increase or decrease the percentage germination of mature *B. punicea* seeds, even at the highest concentrations.

- The undiluted smoke extract (100% smoke) significantly inhibited germination of immature seeds.
- The mature seeds reached maximum germination percentages sooner than the immature seeds.
- The seeds that reached maximum germination the fastest were all grown in dark conditions.
- The most successful treatment in the smoke solution experiment (for mature seed) was 50% smoke (dark), where 100% of seeds germinated in 58 days.
- When *B. punicea* seeds were grown on MS medium there were no significant differences between germination percentages of seeds grown in the light and the dark.
- When seeds were grown on EC medium, both mature and immature seeds germinated better in the light.
- For mature seeds, the highest germination percentages occurred when seeds were grown on MS media (significantly higher than results on EC media).
- For immature seeds, the highest germination percentages occurred when seeds were grown on EC media (significantly higher in the light).
- The mature seeds reached maximum germination faster in the dark than the light.
- The immature seeds reached maximum germination faster on EC medium than MS (in both light and dark conditions).
- In the light and dark experiment, the time range for all treatments to reach maximum germination was smaller for the mature seed (41 - 86 days) than the immature seed (41 - 107 days).
- The addition of the cytokinin, kinetin, to an MS medium appeared to generally increase germination when compared to the control (n.s.).
- Three of the four treatments in which seeds reached 100% germination (in the kinetin experiment) were supplemented with kinetin.

- The fourth treatment in which seeds reached 100% germination contained another cytokinin, 2iP, together with a 1/2 strength MS medium.
- The best media treatment in the kinetin experiment was MS + 128μM kinetin - 100% of seeds germinated in 132 days and the proportion of surviving seedlings was very high.
- Mature seeds from the SC1 seed lot began to germinate 37 DAS *ex vitro* and 36 DAS *in vitro*. Mature seeds from a different seed lot took 52 days to begin germinating *in vitro*.

The natural germination percentage of *B. punicea* seeds is very high, and there does not appear to be any need to pre-treat it before sowing. None of the treatments tested significantly increased the germination compared to the control, and in two of the experiments the control seed actually reached 100%, so could not be improved upon anyway. The only aspect regarding germination in *B. punicea* that could perhaps be improved is the time taken to begin germinating. However, when compared to other species, such as *D. tasmanica*, the time is actually not that long.

Germination experiments comparing different temperatures may be useful as all species have an optimum temperature for germination. Above and below that temperature germination will be delayed or depressed, but not prevented (Mott and Groves, 1981; Bellairs and Bell, 1990). Therefore the optimum temperature for germination of *B. punicea* seeds may speed up the germination process. It may also be worth investigating kinetin treatments in combination with dark, as germination may be even more rapid.

2.5.1.2 *Dianella tasmanica*

- The natural germination pattern of *D. tasmanica* is slow and sporadic and only a low proportion of seeds will germinate, suggesting that dormancy mechanisms are present.
- Control seeds took 114 and 125 days to start germinating.
- Only 38% of control seeds germinated in both experiments.

- The maximum germination of *D. tasmanica* seeds was not reached until more than 500 DAS.
- A dry heat treatment at 50°C did not increase the germination percentage above that of the control. Only 38% of seeds germinated in both treatments.
- Exposure to high dry heat temperatures, above 80°C, for 10 min will kill *D. tasmanica* seeds.
- The oven 50°C treatment caused seeds to germinate more rapidly early in the experiment, but did not change the sporadic nature of germination.
- A fermentation treatment increased the percentage of seeds that germinated above that reached by the control, but the difference was not significant.
- Fermentation caused *D. tasmanica* seeds to begin germinating later than the control seeds, but at the time they began to germinate the number of control seeds that had germinated was actually very similar to the number of fermented seeds.
- Smoke treatments generally increased the percentage germination of *D. tasmanica* seeds compared to the control.
- A direct smoke treatment produced a higher germination percentage (78.3%) than smoke extract solutions.
- The most successful smoke extract concentration was 50% smoke (66.7% of seeds germinated).
- Direct smoke and 50% smoke treatments were not significantly different to each other but produced significantly higher germination percentages than all other treatments (with the exception of 10% smoke).
- The undiluted smoke extract (100% smoke) significantly inhibited germination of *D. tasmanica* seeds (only 11.7% germinated).
- 35% of the control seeds in the *D. tasmanica* smoke experiment germinated.
- The percentage germination of *D. tasmanica* seeds increased with increasing smoke extract solution (with the exception of 100% smoke).

- Seeds from the direct smoke treatment began to germinate before the control and smoke extract seeds (65 DAS).
- Seeds from the 100% smoke treatment were the slowest to begin germinating (128 DAS).
- Smoke treated seeds (both direct and in solution) began to germinate sooner than the control seeds (with the exception of 100% smoke).
- The control seeds began to germinate 121 DAS; 50% smoke, 72 DAS; 10% smoke, 86 DAS; and 1% smoke, 114 DAS.
- *D. tasmanica* seeds from the smoke experiment took between 191 and 500+ days to reach maximum germination.
- Direct smoke treated seeds reached maximum germination fastest (191 DAS).
- The control and 100% smoke treated seeds were still germinating 500 DAS.
- The fastest smoke extract treated seeds to reach maximum germination were from the 50% smoke treatment (226 DAS).
- Partial removal of the testa followed by growth *in vitro* significantly increased the germination percentage of *D. tasmanica* seeds compared to the control seeds.
- When the testa was partially removed 90% of *D. tasmanica* seeds germinated *in vitro*.
- *D. tasmanica* control seeds failed to germinate *in vitro*.
- Partial removal of the testa followed by growth *in vitro* also reduced the slow and sporadic nature of germination in *D. tasmanica*; maximum germination (90%) was reached in only 32 days.

The slow and sporadic pattern of germination seen in *D. tasmanica*, coupled with the very low natural germination percentage, would be totally unacceptable as a commercial method of propagation. The oven and fermentation treatments tested did not increase germination percentages either at all or significantly, but both may be worth pursuing. In the oven experiment only three different temperatures were tested. The 50°C treatment did not increase germination above the levels of the

control seed, and the 80°C and 100°C treatments were too hot and actually killed the seeds. It may be worthwhile to test temperatures between 50°C and 80°C, which may be hot enough to weaken or crack the hard seedcoat without killing the seeds. A boiling or hot water treatment may also be worth trying, as wet heat treatments are often more successful than dry heat treatments (Ralph, 1994). However, due to the high temperatures killing *D. tasmanica* seeds in the oven experiment, a hot water treatment may be less damaging than a boiling water treatment. Other treatments which may be useful in overcoming the physical dormancy imposed by the hard seed coat of *D. tasmanica* include: manual scarification - nicking, chipping or piercing the seedcoat; sandpaper scarification and chemical scarification - using strong acids or other chemicals (see Section 2.1.1.1.3 for further information) to abrade the seed coat.

The fermentation treatment was promising as it did actually increase the percentage germination above that reached by the control seeds (although this difference was not significant). A significant increase may have occurred if the seeds were actually leached in running water following the fermentation period rather than just rinsed quickly. A longer leaching period would perhaps have removed the chemical inhibitor faster. It may also be worthwhile attempting a leaching treatment on its own, as this may be enough to remove inhibitors. Soaking the seed in cold water for up to 3 days (with frequent water changes) has been enough to remove chemical inhibitors from seed of some species (Ralph, 1994) and may be worth trying with *D. tasmanica*. Soaking in an alkaline solution (pH 9) could also be successful. A kinetin treatment, which increased germination of *B. punicea* and *D. latifolia* (but not significantly), may also be useful in removing chemical inhibitors, and would be worthwhile trying with *D. tasmanica*. Other chemicals such as thiourea and sodium hypochlorite have also been used successfully to remove chemical inhibitors (Ralph, 1994), and are also worth investigating.

The smoke treatments were very promising, especially the direct smoke treatment which caused the seeds to germinate faster than untreated seeds and produced a higher percentage germination than the oven and fermentation experiments. It would be worthwhile to test a number of different exposure times to direct smoke, as the

2.5-3 hr exposure time was perhaps quite short compared to a natural bushfire situation, in which the burnt vegetation may smoulder for days or even weeks. A longer exposure time may further increase the percentage germination and/or decrease the time to germination. As only four different smoke extract concentrations were tested in this experiment, it would also be worth investigating a wider range of concentrations, especially between 50% smoke (which elicited the best germination response in the current experiment) and 100% smoke (which inhibited germination). It is possible that a concentration within this range may increase the percentage germination even further, and/or further decrease the time to germination. As smoke has produced an additive effect when used in combination with other treatments (such as heat, dark or scarification), this approach would also be worthwhile investigating for *D. tasmanica.* Dark conditions (often in combination with smoke treatment) promoted more rapid germination than light for both *B. punicea* and *I. tasmanica.* Therefore, an investigation into the effects of dark on germination of *D. tasmanica* seeds would also be warranted.

The partial removal of the testa, followed by growth *in vitro* was the best treatment investigated for this species. However, as *in vitro* techniques can be expensive, it would be worth determining the success or otherwise of this treatment under *ex vitro* conditions. As the removal of the entire seedcoat has been so successful for other species (see section 2.1.1.2.5), it would also be worthwhile to try this method for *D. tasmanica*, as this may further increase the rate and/or percentage germination above that achieved by partially removing the testa. However, this may be difficult to achieve without damaging the embryo.

2.5.1.3 *Milligania densiflora*

- Older seed which had been in storage at the MBG failed to germinate, suggesting that fresh seed should be used.
- The germination percentage of *M. densiflora* control seeds was higher *in vitro* than *ex vitro* (65% and 88% *in vitro* compared to 48% *ex vitro*).

- *M. densiflora* seeds began to germinate quite quickly, 16 - 17 DAS, both *in vitro* and *ex vitro*.
- Under *ex vitro* conditions, seeds in the warmer incubation room reached a significantly higher percentage germination than those in the laboratory.
- The rate of germination was initially more rapid in the incubation room compared to the laboratory.
- A disinfestation period of 15 min in 2 % NaOCl was not very successful for *M. densiflora* seeds, with a high percentage of contamination occurring.
- *M. densiflora* seeds should be disinfested for at least 35 min in 2% NaOCl, and perhaps longer, to reduce contamination levels.
- Contamination rates were reduced significantly (by approximately 50%) when seeds were disinfested in 2% NaOCl for 35 min compared to 20 min.
- When seeds were disinfested for 20 min in 2% NaOCl the percentage germination was similar, regardless of media type.
- Germination percentages were higher when seeds were disinfested for 35 min compared to 20 min, regardless of media type.
- The percentage germination was highest in the 1/2 MS, 35 min disinfestation treatment (90%). This treatment was significantly different to all other treatments.

The results from experiments on *M. densiflora* seeds have suggested that NaOCl may be acting as a scarification agent, and this should be studied further. The use of the chemical at a higher concentration may further increase germination, so an experiment testing different concentrations (from 2% to 5% or even 10%) with a range of exposure times would be worthwhile. Other chemical treatments, such as kinetin, could also be tested.

Although the seed coat does not appear to be exerting any physical dormancy on the seed (with seed taking only 16 - 17 days to begin germinating) mechanical scarification agents such as sandpapering the testa, or nicking the seed coat, may

increase the percentage germination. The warmer temperature in the incubation room increased the percentage germination, so it may also be worthwhile testing the germination at a range of temperatures. Higher temperatures of 30°C or above, which would often be experienced in alpine areas in summer, may further increase the germination of this species.

2.5.2 Iridaceae species

2.5.2.1 *Diplarrena moraea*

- *D. moraea* seed must be fresh or the viability will be significantly reduced.
- The natural germination percentage of fresh seed is very high (97 %).
- To successfully disinfest seeds for *in vitro* germination experiments, seeds must be immersed for at least 15 min in 2 % NaOCl.
- A weaker 1 % NaOCl solution was not very successful.
- Seeds began to germinate quite quickly (10 DAS) and also reached maximum germination quite quickly, with the majority of seed germinated by 51 DAS.

The natural germination rate of *D. moraea* seeds was very high, suggesting that propagation by seed would be commercially viable. As such, it is probably not worth pursuing other treatments. However, it may be worthwhile to treat seeds with different concentrations of kinetin, as this chemical increased the natural germination level of the closely related *D. latifolia* (but not significantly). As *D. moraea* inhabits fire-prone areas, and flowers prolifically following fires (Conn, 1994) a smoke treatment may also be beneficial.

2.5.2.2 *Diplarrena latifolia*

- The natural germination percentage of *D. latifolia* seed was reasonably high at 76 %, but not as high as *D. moraea*.
- When germinated *in vitro*, seeds on MS medium reached a higher germination percentage than those grown on EC medium (only significantly different between MS light and the EC treatments).
- Seeds reached a higher germination percentage in the light than in the dark (n.s.).
- Seeds also began to germinate sooner in the light than in the dark.
- The presence of a high concentration of kinetin in the medium also sped up the germination process.
- *D. latifolia* seeds took between 94 and 158 days to reach maximum germination; the control treatment took the longest.
- Treatments with the highest germination percentages, MS + 128μM kinetin and MS + 8μM kinetin, reached maximum germination quite early within the range (111 and 117 DAS, respectively).
- In the majority of treatments a small proportion of seedlings died soon after germinating.
- Taking all measured factors into account, the best treatment for germination of *D. latifolia* was MS + 128μM kinetin.

The natural germination rate of *D. latifolia* is reasonably high, and probably high enough that seed germination could be used as a commercial propagation method, without the need for any pre-treatment. The successful use of kinetin to increase this natural germination percentage prompts the suggestion that other chemicals may do the same; and these may be worth trying. For example, other cytokinins, such as 2iP, which produced a similar effect to kinetin on *B. punicea* seeds, or chemicals such as thiourea which have been used successfully with other species (Ralph, 1994). A smoke treatment may also be successful.

2.5.2.3 *Isophysis tasmanica*

- *I. tasmanica* seeds germinate very well without a seed pre-treatment, reaching 90 to 100% germination in control treatments, suggesting that there are not any dormancy mechanisms affecting germination of these seeds.
- The treatments that produced the highest germination of 100% were: control dark, 10% smoke dark, 50% smoke light, and 50% smoke dark.
- Germination percentages were significantly higher in the dark than the light *in vitro*, but there were no significant differences between the dark and light *ex vitro* treatments.
- Seeds began to germinate faster, and also reached maximum germination faster in the dark than the light both *in vitro* and *ex vitro*.
- The first seeds to germinate took 48 days *in vitro* and 52 days *ex vitro*, and between 94 to 173 days to reach maximum germination.
- Aqueous smoke solutions do not improve germination of *I. tasmanica* seeds, and actually inhibit germination when used undiluted (n.s.).

As the natural germination percentages of *I. tasmanica* are very high it is probably not worth pursuing any treatments to increase this level of germination. However, the time taken to begin germinating and to reach maximum germination could perhaps be improved upon. A kinetin treatment may be worth trying, as this chemical (particularly at high concentrations) had positive effects on the germination of *B. punicea* and *D. latifolia*. Experiments comparing a range of temperatures would also be worthwhile, as germination rate would be faster at the optimum temperature for germination of *I. tasmanica* seeds.